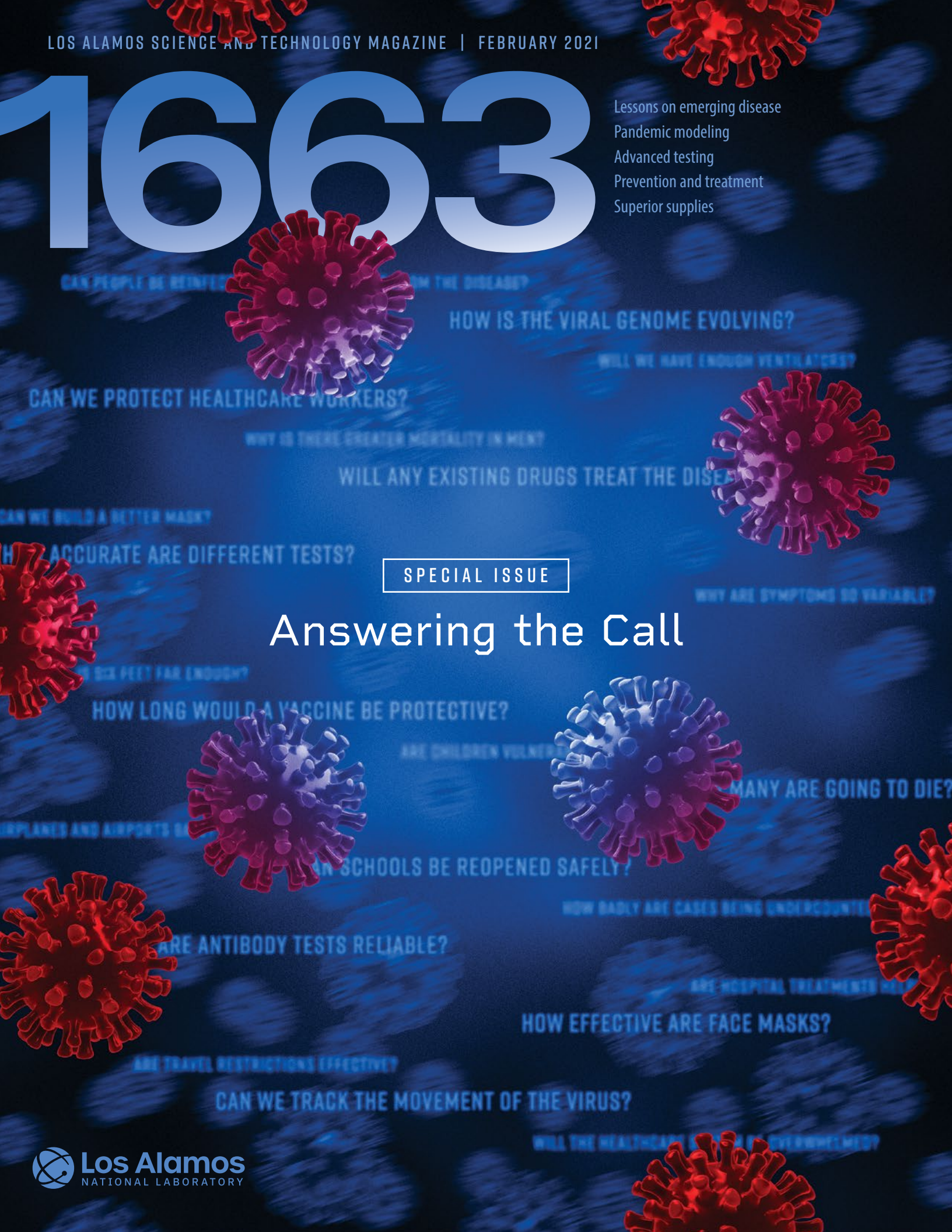
LOS ALAMOS SCIENCE AND TECHNOLOGY MAGAZINE | FEBRUARY 2021

1663

Lessons on emerging disease
Pandemic modeling
Advanced testing
Prevention and treatment
Superior supplies



SPECIAL ISSUE

Answering the Call

Los Alamos NATIONAL LABORATORY

During the coronavirus pandemic, there has been some debate about the relative effectiveness of various face coverings. Without data it can be hard to know which materials work best and in what circumstances. Laboratory scientists have experimentally determined that cloth masks of nearly any kind do help limit the spread of the virus. As part of their investigation, the scientists produced scanning electron microscope images of different materials, including the central filtering layer of a surgical mask, the central filtering layer of an N95, a single layer of quilters cotton, and a single layer of a bandana. Surgical masks and N95s have multiple layers of different materials, which have each been engineered to do a particular job, while homemade masks typically include two or more layers of a single material. For more about masks and virus-containing respiratory droplets, see "New Tools for the Toolbox" on page 28.

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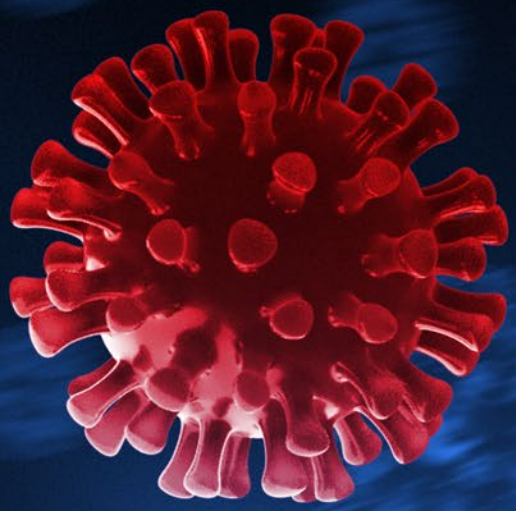
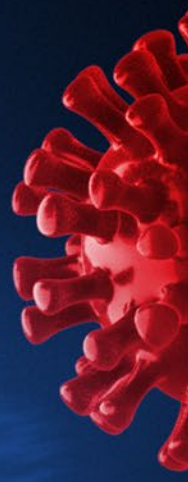
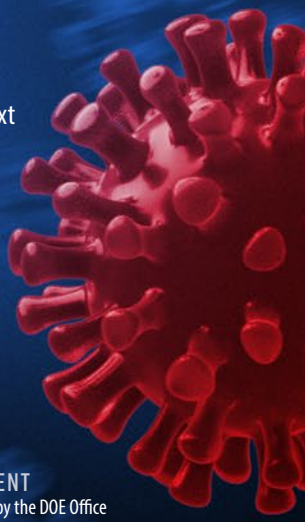


THE WORLD HAS CHANGED. For the first time in a century, a massive worldwide pandemic threatens both our lives and our way of life. There can be little doubt that this crisis calls for technological solutions and scientifically informed policies, and the national laboratories of the United States have an essential role to play. The federal government created the National Virtual Biotechnology Laboratory to coordinate the national laboratories' efforts and capitalize on their expertise to address everything from pandemic modeling and computing to patient testing, the development of therapeutics, and the manufacture of critical supplies.

Over the past several decades, Los Alamos National Laboratory had the foresight to develop many of the key capabilities the world needs right now. The Laboratory has long been on the forefront of high-performance computing and complex-system modeling, which now provide the basis for predicting the course of the pandemic and virtually screening thousands of potential drug candidates. It has long been on the forefront of genomics as well, which helps reveal the virus's targetable proteins and track viral mutations across the population. It has long been on the forefront of personal protective equipment and worker safety due to its experience with radioactive and hazardous materials. It has analyzed the motions of gases, droplets, and particulates; developed diagnostics and monitoring systems for biothreats; and even designed a new kind of HIV vaccine, currently undergoing clinical trials, expressly for the purpose of protecting against a fast-evolving virus. Examples of hard-won, pandemic-relevant Los Alamos expertise abound, and the Laboratory was able to quickly redirect these capabilities to the crisis at hand.

In the pages that follow, we present a subset of the comprehensive array of research Los Alamos scientists are carrying out to protect us all.





ABOUT OUR NAME

During World War II, all that the outside world knew of Los Alamos and its top-secret laboratory was the mailing address—P. O. Box 1663, Santa Fe, New Mexico. That box number, still part of our address, symbolizes our historic role in the nation's service.

ABOUT THE LDRD LOGO

Laboratory Directed Research and Development (LDRD) is a competitive internal program by which Los Alamos National Laboratory is authorized by Congress to invest in research and development that is both highly innovative and vital to national interests. Whenever *1663* reports on research that received support from LDRD, this logo appears at the end of the article.

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One Planet, One Health

Biologist **Jeanne Fair** knows that the way to anticipate pandemics is through understanding the connections between humans, animals, and the environment.

LAST FEBRUARY, WHEN COVID-19 WAS JUST BEGINNING to visibly emerge from China, I was asked to participate in a Laboratory panel discussion about the spread of this novel coronavirus and what it might mean for the United States. Having studied emerging zoonotic diseases for 25 years, I knew the outlook was troublesome, but I was still optimistic. I had a good idea of the extensive disruption that was coming our way, and yet, when the situation escalated dramatically a month later, I still found it hard to believe and harbored a strong sense of denial.

Anytime I've given a public lecture on emerging diseases and pandemics, I have shared a photo of a gravestone in Kansas that belongs to my great-aunt, Margret Ann Fair, who died of the 1918 influenza at the age of four. I often read her obituary to make the point that likely everyone in the room has an ancestor in their family tree who died during the 1918 pandemic. Like most researchers who study emerging infectious diseases, I always said that it was not a question of "if" a new disease could become a pandemic; it was instead a question of "when."

And yet, there are days that I still can't believe it is actually happening to us right now.

How did we know?

The reason I said it's a question of "when" is because it is not uncommon for new diseases to emerge. Pathogens that can infect multiple species—such as rabies, influenza, and Ebola—are everywhere. Infectious diseases frequently spill over from animals to humans and vice versa, but we don't always notice. Sometimes the pathogen is not particularly virulent, meaning it does not cause severe disease. Other times a pathogen may infect a "dead-end host"—one that does not transmit the pathogen further.

The key to a spillover becoming an international crisis is a combination of transmissibility and virulence. Avian flu (H5N1), for example, is extremely virulent and can make a person deathly ill in a short amount of time, killing about half of the humans it infects. This is scary, but with known pathogens of pandemic potential there has often been a tradeoff between virulence and transmissibility. The H5N1 avian influenza virus attacks us deep in the lungs and therefore is not as transmissible as seasonal influenza, which replicates more in the upper respiratory system. On the other hand, common colds—many of which are caused by coronaviruses—are extremely transmissible but not very virulent.

Infectious diseases frequently spill over from animals to humans and vice versa, but we don't always notice.

Severe Acute Respiratory Syndrome (SARS), which emerged in 2002, is a deadly disease caused by SARS-associated coronavirus (SARS-CoV). This was the first severe and readily transmissible disease to emerge in many decades; it quickly spread to more than 20 countries, infected about 8000 people, and killed 774. In 2012, a related coronavirus emerged as a much more deadly disease. Middle East Respiratory Syndrome coronavirus (MERS-CoV) infected about 2500 people and caused 858 deaths. Fortunately, MERS was also not particularly transmissible and was quickly controlled.

Today's pandemic is caused by SARS-CoV-2. It is closely related to SARS-CoV and MERS-CoV, but it had the correct mix of transmissibility and virulence to take the world by storm. With SARS-CoV-2 there are no tradeoffs: it is easily transmitted between people and it is very deadly in some. But it is that extreme variability in the disease characteristics and virulence in humans that adds to the perfect storm. SARS-CoV-2 can take from five short days to two entire weeks to incubate in a host before it causes coronavirus disease 2019, COVID-19 for short. But in some people, it never shows itself. Many who are infected with SARS-CoV-2 do not

In some parts of the world, people buy food from live meat markets, including bats and other wildlife. Wildlife in cages in these "wet markets" are under stress, which makes them more vulnerable to disease than they might be in the wild. Wet markets are one way that animal diseases can spill over into humans.

experience any symptoms (they are asymptomatic) or experience only mild symptoms—but they can still transmit the virus. The potentially long incubation period and pre-symptomatic or asymptomatic spread are some of the reasons this virus is so serious and also so complicated. All of this variation in the disease and transmission leads to variation in human behavior and in our response to the pandemic. This variation is something we are all witnessing every day.

It is not possible to predict exactly when a mutation will happen to make a pathogen pandemic-worthy or to anticipate when a spillover will occur. However, scientists know there are situations that can increase the likelihood of a spillover event, such as changes to the environment and climate or significant interactions between humans and wildlife (e.g., eating bushmeat or going to live meat markets). Scientists have combined these attributes to predict hotspot areas for emerging diseases. There is also a lot that we do know that can help with prediction. If we know which specific pathogens have pandemic potential, and which animals harbor those pathogens—we call them disease reservoirs—then we can monitor them as part of our global biosurveillance effort.

SARS-CoV-2 most likely originated in bats. Bats and humans—and other animals like cats—have angiotensin-converting enzyme 2 (ACE2) attached to the outsides of their nasal, lung, and kidney cells. These ACE2 enzymes normally serve as docking stations for a protein called angiotensin that helps regulate blood pressure, wound healing, and inflammation. SARS-CoV-2 binds to ACE2 as a way to gain entry into cells. This is how diseases can jump between species: if humans share certain receptor molecules with animals, then pathogens that use those receptors can spread to humans.

Humans in some cultures eat wildlife, and often keep them in live meat markets where refrigeration is not possible. Wild animals in cages in these "wet markets" are under stress, which makes them more vulnerable to disease than they might be in the wild. And this is how spillover happens: a person is exposed to an infected animal and if the pathogen has new mutations or is novel to that person's immune system—and if it has the ability to dock and enter the person's cells—the emergent pathogen could infect the person. If this happens, the pathogen could thus take hold in a new species.

The "One Health" paradigm is the concept that the environment, wild animals, agriculture animals, humans, and pathogens are interconnected. It is important to understand each of these compartments and their connections to ensure the health of all populations on the planet.

The ecology of infectious diseases is something I have been studying for the last 25 years. I have been fortunate to have met the top scientists around the world who study infectious diseases and spillover events. These researchers are some of the most dedicated and hardworking scientists anywhere, and their efforts are now focused on helping the global community understand why this pandemic happened and what to do before the next one.

Studying wing feathers is an important part of disease biosurveillance in birds to assess their health, condition, and age. This is valuable for building population models for species and for assessing the impacts of mass mortality events and the relative risk of new spillover diseases. *CREDIT: Jeanne Fair/LANL*

Canaries aren't the only harbingers

I first came to Los Alamos to do my graduate work on western bluebirds. I became interested in zoology as an undergraduate—giving up my previous plans to study bass violin—when I realized I just couldn't stop thinking about the natural world. During my time as a graduate student at Colorado State University I worked on a United States Department of Agriculture project on the impact of insecticides on birds. I became interested in toxicology and understanding the effect chemicals have on the immune system and other physiological processes that make an animal more susceptible to infectious disease.

My graduate work with the Environmental Restoration project at the Lab was an opportunity to set up a network of nestboxes across the Pajarito Plateau (upon which Los Alamos sits) with which to study wild bird individuals and populations. We wanted to determine if stresses such as legacy environmental pollution, food limitation, habitat changes, or climate shifts were impacting the birds in the region. With the nestboxes, we could take physiological measurements of the birds that were surviving and breeding, but to thoroughly answer our questions we needed a way to measure the birds' immune systems. The year 2020 was the 24th field season for the Los Alamos Nestbox Network (now operated by the Lab's Environmental Stewardship Group), and I am grateful to the teams of students and staff who have dedicated their time and passion into keeping this project going. As it turns

Los Alamos researchers have found that tree mortality in the Southwest is caused by a combination of drought, higher temperatures, and bark beetles. These habitat changes have been documented by Jeanne Fair and her team to have drastic consequences to bird populations, including impacting their immune systems and increasing their risk for carrying disease. Deforestation for agriculture and development all over the world also destroys animal habitats. Such circumstances can lead to pandemics when changes in habitat bring wild animals under stress into closer contact with humans.

out, long-term studies are how we can answer these difficult questions.

One of the things that attracted me to Los Alamos—and kept me here—is the innovation in biotechnology and modeling. For example, the invention of flow cytometry (a tool for evaluating and sorting cells) and the seminal work in genomic sequencing both happened here. I saw an opportunity to apply these tools to novel systems, such as wildlife and infectious-disease surveillance. My colleagues Kirsten McCabe, Babetta Marrone, and I worked to develop flow cytometry-based immunological assays to detect antibodies in different species of birds because most tests had been developed for chickens and wouldn't work on other species. In 2003, when New Mexico was hit the hardest by West Nile Virus, we lost 98 percent of the black-billed magpies in the Pojoaque valley below Los Alamos. This project led us to explore a central question to understanding infectious diseases: Why are some species susceptible while others are dead-end hosts?

Species susceptibility is one aspect of studying emerging diseases, as it helps focus surveillance efforts on the appropriate animal populations. Furthermore, it is critical to appreciate all the factors that can make an animal stressed, which also adds to its susceptibility. Stress is a physiological response in the body, and some stress hormones, like cortisol, are known to suppress parts of the immune system, such as the inflammatory response. Being in a cage at a wet market is one source of stress for an animal, but there are many others. Deforestation and other human activities lead to habitat loss, forcing animals to move to new areas where they may face food insecurity or new predators. Biodiversity in areas around the world is a good index of a healthy system; it is often inversely related to the occurences of emerging zoonotic diseases and outbreaks. Environmental changes, especially to the climate, can also lead to forced migration of animals. Finally, environmental pollution, as we have long been studying in Los Alamos, can be a source of stress if it causes damage to animals' immune systems or reproductive systems.

Using the Los Alamos Nestbox Network, our results showed that legacy environmental contamination had little impact on the birds. However, we observed large impact to the birds from habitat changes. The increased temperatures in Northern New Mexico and resulting tree die-off at lower elevations forced the birds to move to higher elevations. Furthermore, in the drought year of 2002, nestling bluebirds had half the immune system capacity compared to a normal year.

Recently, we observed an example of multiple stresses combining to cause a mass wild-bird mortality event. In September 2020, an estimated one million birds died in New Mexico and southern Colorado, where they had been experiencing a combination of drought, wildfire smoke, and weather extremes (temperatures close to 100 degrees Fahrenheit one day and snow two days later). Because of this event, we are creating a Southwest Avian Health Network to connect ornithologists in New Mexico to those in neighboring states.

As my work progressed over the years, I began to focus my attention on measuring other signatures in birds to help with assessing disease susceptibility. Some of my studies focused on a sugar molecule called sialic acid, which is the receptor for influenzas. Knowing that avian influenza is highly pathogenic in chickens but causes minimal disease in other bird species, we sought to determine if different birds have varied amounts of sialic acid, which could make some more susceptible, or even superspreaders, of disease. In a recent publication, we demonstrated that sialic acid does indeed differ among bird species and that some blood cells in birds had more human-type sialic acid than expected. This is an important finding for birds, since sialic acid is also a receptor for the parasite *Plasmodium*, which causes malaria in birds and humans.

SARS-CoV-2 had the correct mix of transmissibility and virulence to take the world by storm.

So many systems

To this day, I continue my work studying bird species' susceptibility to disease and biosurveillance in animals. However, in 2004 I also began broadening my scope and looking at pandemic modeling as part of a Department of Homeland Security project on H5N1 avian influenza. Our project focused on modeling the impact of a pandemic on critical infrastructures. We looked at hospital capacities, the impacts of closing schools and limiting contacts, and the effectiveness of targeting treatments if available. (With influenza, we knew that Tamiflu could help but that we would have limited doses.) In 2009, we were part of a multi-team effort at the Laboratory using several of our epidemiological models to predict the impacts of different scenarios for pharmaceutical and vaccine interventions for the spreading H1N1 influenza ("swine flu") pandemic. It was exciting to work with the amazing scientists and epidemiologists at Los Alamos, and it was a true collaborative effort.

It was during my eight years with this critical infrastructure modeling team at Los Alamos, and with two Laboratory-directed exploratory research projects for understanding both West Nile Virus and avian influenza host heterogeneity, that I fell in love with scientific collaboration. While the science was engaging and interesting, it was collaboration with my colleagues that most excited me. I began to seek out information to learn how to make collaborations better and how to foster collaborations between my fellow researchers. I became enthralled with the emerging field of the "science of team science" and what makes transformative science teams. I sought everything I could read on leadership, communication, and teamwork. This interest would pay off later when I did an assignment in Virginia as a Biological Threat Reduction Program (BTRP) Science Manager with the Defense Threat Reduction Agency.

Although genomic sequencing technology was advancing at a rapid pace in the early 2000s, enabling scientists to evaluate the blueprints of organisms and thoroughly understand how

they are related and how they differ, its full potential could only be realized via strong international partnerships. When it comes to pathogens, the genetic blueprint is vital, and current sequencing technology makes it possible to study a genome relatively quickly. As we see today with COVID-19, most testing is based on reverse transcriptase polymerase chain reaction (RT-PCR), which matches pieces of the viral RNA from a patient to known SARS-CoV-2 sequences.

This pandemic is transformative, pushing science and collaborations to their highest potential.

Accessing sequence data from around the world is critical to our ability to understand biological threats such as SARS-CoV-2 and others. This data availability, however, depends on two things: that other countries have the technology to provide such data and that we have cultivated relationships with them so that we can easily collaborate. In 2011, Los Alamos began participating in two programs to promote international scientific engagement: the BTRP and the Department of State's Cooperative Threat Reduction (CTR) program. Through these programs, Los Alamos scientists have helped establish sequencing centers in multiple countries (including the Republic of Georgia, Jordan, Kenya, and Uganda), and my amazing colleagues continue to support more than 29 countries worldwide with ongoing sequencing and bioinformatics training.

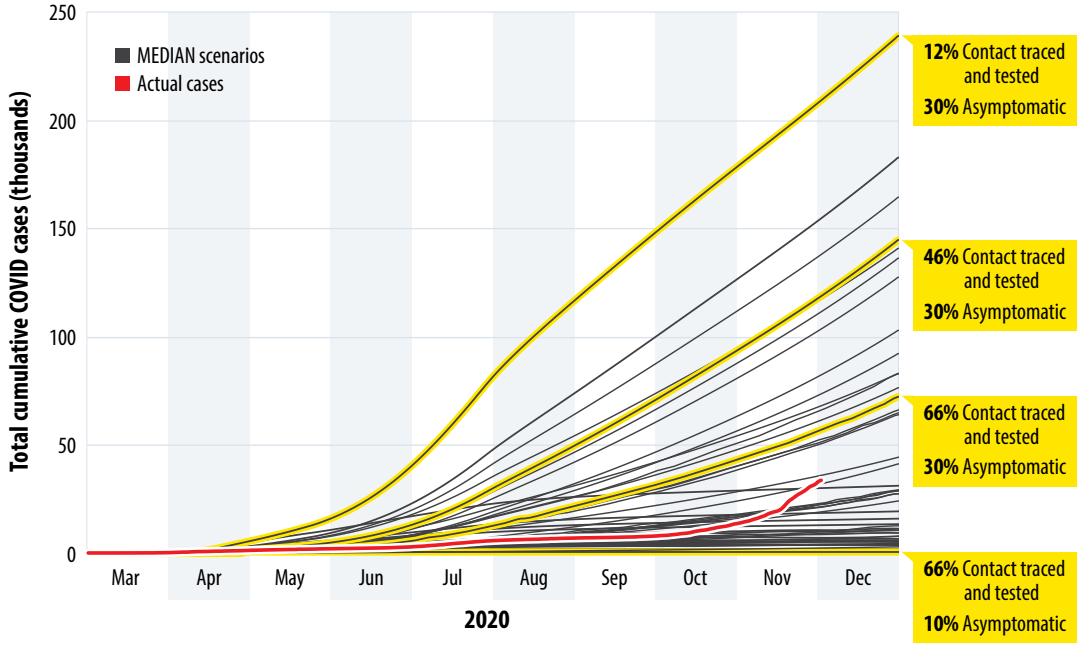
I believe these cooperative-engagement programs comprise some of the most important international efforts by the United States. In my role as a BTRP Science Manager (2013–2016), my job was to help the United States develop collaborations with partner countries to build their capabilities in detection, diagnostics, and reporting. As a result, biosurveillance and infectious disease technologies were strengthened in these partner countries, and we created strong, lasting relationships through scientific diplomacy and building trust between countries. Through this partnership, diagnostic laboratories around the world were better prepared to respond to the COVID-19 pandemic and provide ample and accurate diagnostic testing for their citizens.

When "if" becomes "now"

It is strange to spend decades studying something you hope won't happen, but here I am. This pandemic was accurately predicted—we knew the hotspots where a spillover would most likely occur, and we knew that coronaviruses could spill over. We also knew what to do: stamp it out when it starts, test, contact trace, and quarantine. Yet here we are.

Since February, I have spent my time focusing on two main areas. First, my colleagues and I are supporting our BTRP partner countries by sharing best practices both to improve COVID-19 sequencing and genomics in their local laboratories and to help feed the international databases with valuable sequences. Second,

Evaluating the impact of contact tracing and testing



The epidemic curves in the graph represent different scenarios for the region of Albuquerque, NM. Each scenario comprises multiple variables for controlling the pandemic—such as diagnostic testing time, false-negative rates, and fractions of individuals who were contact traced and quarantined. Scientists can then quantify the extent to which the variables are effective at lowering case numbers. Statistical analysis of the scenario data indicates that contact tracing and quarantining have a consistently large impact on slowing disease spread, although these countermeasures are difficult to implement effectively if a large fraction of cases are asymptomatic. The red line shows actual cases throughout New Mexico, which were kept down by the implementation of many strategies, including widespread contact tracing and testing.

As part of her graduate work, Jeanne Fair set up a network of 400 nestboxes across the Pajarito Plateau. Taking samples from birds in these nests provides data about how stresses, such as environmental pollution, food limitation, habitat changes, and climate shifts, impact birds in the region. The year 2020 was the 24th season for the Los Alamos Nestbox Network.

we reconvened our critical infrastructure modeling team, gave it a new name, and have been ferociously working on developing a modeling experiment to understand the uncertainty of the pandemic. Now called MEDIAN (Modeling Epidemics for Decision support with Infrastructure ANalysis), this systems-dynamics model includes a range of distributions for all aspects of what is going on, from disease characteristics (incubation, pre-symptomatic transmission, mortality rate) to the uncertainty of human behavior (quarantining, wearing masks, rates of contact tracing).

Using MEDIAN, we are looking to identify the most important factors contributing to the severity of the pandemic. Our model is not about predicting how many people will be infected tomorrow or will die next week—other Los Alamos models examine this important aspect (see *What Happens Next* on page 10). Instead our goal is to take into account the uncertainty and variability in the pandemic to help understand which things are driving its spread and where to focus our response.

This understanding is derived from specifically designed experiments where we evaluate various distributions and look for key correlations. For instance, we might design an experimental run that has a disease incubation period of 5–14 days, with a percentage range of pre-symptomatic transmission, an age distribution of mortality rates, and a few other variables, each with a lot of uncertainty. Then we can run 10,000 simulations overnight covering all the uncertainties and we get a spaghetti figure of epidemic curves that is not that helpful. However, once we have this spaghetti figure, we can use statistics to pull out the most important disease and mitigation variables driving the epidemic. For the COVID-19 effort, we are focusing on the role of diagnostic testing. This will help us investigate the different patterns associated with testing strategies. For instance, we are seeking to correlate the impact of mass testing with contact tracing and quarantine on the number of cases and deaths.

One day at a time

This is hard. There is still so much uncertainty with this pandemic, and it can be overwhelming. However, there is much we *do* know that can help us today and that will help us prepare for tomorrow and the next emerging disease. Our work on COVID-19 is immediate and critical, but the rest is still important.

Our small lab group in Bioscience Division is looking at the big picture of emerging disease by taking into account all aspects, such as climate change, environmental change, biodiversity impacts, and plant pathogens, as part of our Ecological Health Security Lab. For example, understanding how long-term environmental change impacts future mosquito distributions and the infectious diseases they carry is one important aspect. To address this, we are part of a new Laboratory-directed project to couple Earth systems and epidemiological models; this infectious disease-climate team is a true collaboration across many directorates and divisions at Los Alamos.

Ecological health security is the epitome of a complex system. Our ecology is changing: our proximity to and relationships with animals are evolving, our climate is adjusting, and the plants and wildlife are adapting (or not). This pandemic will not be the last, but hopefully it will be the worst—because we will learn from it and prepare. The conditions are ripe for new diseases to catch us off guard, but we have the technology and the partnerships to respond. This moment is transformative, pushing science and collaborations to their highest potential.

I still have moments where the reality of COVID-19 hits me hard—we never thought it would be this bad. But I try to stay positive and be grateful each day for nature, for the people in my life, for living in our amazing state of New Mexico, and for my health. I also know that tough times never last, but tough people do! And to be honest, waking up with a pressing purpose in the morning can be a good thing. LDRD

—Jeanne Fair

MORE DISEASE AND ECOLOGY AT LOS ALAMOS

http://www.lanl.gov/discover/publications/1663/archive.php

- **Disease surveillance and response**
  "Defining the Danger" | March 2018
  "Biosurveillance" | July 2013
- **Tree mortality**
  "Diagnosis: Drought" | October 2017
  "Our Dying Global Forests" | October 2012
- **Bird adaptations**
  "Body Building" | April 2014

from 1663

COMPUTING AND MODELING

WHAT HAPPENS NEXT

Inside the Herculean effort to anticipate the path of the virus and mitigate its impact

"THE OUTBREAK SHOULD FOLLOW THE SAME PROCESS in every community," says Los Alamos scientist Ben McMahon. "At least in theory. It should get worse and worse until the community realizes they have to get serious about isolating, and then it should fall away quickly. The epidemic curve is really a learning curve."

But the COVID-19 outbreak is far from a textbook event, and McMahon, a key player in Los Alamos's comprehensive effort to model the pandemic, is knee-deep in all the ways the learning curve can be distorted. In a joint enterprise to model the pandemic for better-informed policymaking, Los Alamos shares detailed weekly reports with three other national laboratories, and every single week—even after the better part of a year—surprising, fundamental new information is still coming to light. For a disease that stubbornly carves out an exception to nearly every rule the experts try to attach to it—from the symptoms it produces to the effectiveness of the antibodies its survivors retain—McMahon and his colleagues strive to assemble the most believable set of "facts" possible and feed them to a computer to answer one question: What is likely to happen next?

"The trouble is"—McMahon has to interrupt himself here—"well, one of the many troubles is: Susceptibility varies greatly depending on age, sex, and certain preexisting conditions. That means some people are substantially less likely to die, get tested, or even show any symptoms, even though they may be every bit as likely to transmit the virus." With something like Ebola, everyone is suitably terrified, young and old, and the learning curve is very steep: isolate or die. With COVID-19, the weight of the message is considerably more fragmented.

Uncertainties about both the contagion itself and the personal and societal behaviors that contribute to either its spread or its containment greatly complicate researchers' efforts to predict the course of the pandemic. But that information is absolutely crucial. If policymakers know the potential landscape of tomorrow, they will have a much better idea of what to do about it today.

What will happen

Los Alamos has a number of COVID-modeling efforts underway. The most widely shared of these is on its public website and featured on the Centers for Disease Control (CDC) website as well, due to its track record for accuracy. The model spans the globe, country by country, and the United States, state by state. It is produced and managed by a team of about 20 Los Alamos specialists, including computer scientists, bioscientists, mathematicians, economists, and others; statistician Dave Osthus leads the team.

"Our model produces forecasts, not projections," Osthus explains. "Whereas a projection predicts what *would* happen if various strategies were put in place or various circumstances came to pass, a forecast directly predicts what *will* happen based on what is already happening." That doesn't mean it ignores policy interventions, such as stay-at-home orders—far from it. But rather than trying to figure out how much of a difference they *ought* to make, the model examines how much of a difference they are already making or how much difference they have already made elsewhere. The result is an ultimate best-guess at the future—cumulative confirmed cases and deaths—driven by real-world data.

Real-world data, however, are not especially straightforward. Actual cases are sharply different from confirmed cases; confirmed cases result from testing, and testing is not uniformly accurate. And even if all COVID-19 tests were perfectly accurate, there would still be a huge question mark when it comes to who is getting tested. How many people? Which ones? People who are

already sick? People who visit a clinic for some other reason? Or a cross section of the public at large? There is tremendous variation in procedures from state to state and even county to county, since much of this data is obtained by public health departments at the county level. The Los Alamos statistical model has to deal with these challenges and generate the most reliable prediction possible anyway.

Unlike flu, there is no historical data on COVID-19—no benefit of hindsight.

To do that, the model has to learn; it has to assimilate large amounts of data and figure out how to recognize trends, broken down by region. It also has to learn from its mistakes. As events unfold and new data are gathered from one week to the next, the model must attempt to improve itself.

Fortunately, Osthus had already been working with just such a machine-learning model, called Dante, to predict recent flu seasons. In a contest sponsored by the CDC for the 2018–2019 flu season, 24 teams submitted model output, and Dante's predictions came closest to matching reality. Osthus and others reworked it for the COVID-19 pandemic.

However, COVID-19 and flu have two important differences, in terms of modeling. The first is the fact that people have been dealing with the flu for ages, and there is a lot of valuable historical data to work with, but not for COVID-19—there's no benefit of hindsight. All the data on COVID-19 comes from the current pandemic in real time. To put it bluntly, the forecast gets more accurate if more people get sick and die.

The other major difference between the current pandemic and the flu stems from individual behavior. Because flu is so familiar, the range of human behavior is not very wide. A relatively consistent fraction of infected people will go to work anyway, despite feeling sick. A relatively consistent fraction of people will see a doctor. A relatively consistent fraction of people will get a flu vaccine each year. It is through this similarity from season to season that a gigantic source of uncertainty—human behavior—can be tamed. But with COVID-19, individual behaviors are critical, and there is no historical basis to justify anything modelers might assume. Hand washing, face masks, social distancing, restricted travel—such things vary to a large degree and are extraordinarily difficult to predict or even assess after the fact. How often did residents of Hawaii or Ohio wash their hands in the past month? How seriously did they adhere to social distancing mandates?

Without knowing the answers to these kinds of questions, it's difficult to predict the future. It's even more difficult to determine which interventions would be the most effective. But just because individual behavior is difficult to quantify doesn't mean Los Alamos scientists can't find a way to model it.

What would happen

A trio of cause-and-effect, rather than statistical, Los Alamos models is intended to address what-if questions. What would happen if schools ramp up onsite learning? Or if non-pharmaceutical interventions, such as face masks, social distancing, and hygiene measures, were intensified (or reduced)? Or if a vaccine were distributed in a particular way?

Perhaps the most straightforward of these models is EpiGrid, an epidemiological model that tracks the geographic spread of a disease by breaking the landscape into a connected grid of 10-kilometer-square regions, rather than administrative units like countries, states, or counties. Originally developed as a risk-assessment tool for bioterror attacks and natural pandemics, EpiGrid is comprehensive and versatile, making do with imperfect data. Scenarios have been developed for many countries, pathogens, and assumed responses.

EpiGrid accounts for details of the infectious agent itself (How long does it incubate? How is it transmitted—droplets, contaminated water, mosquitoes, etc.? Are asymptomatic or pre-symptomatic people contagious? Can people who have recovered be infected again?), the progression of the disease (How many people are susceptible? Exposed? Infected? Seriously ill or hospitalized? How many have recovered? How many have died?), the modes of treatment (Antivirals? Vaccines? Other treatments?), and societal actions (Are quarantines in place? Are masks required? Are schools open?).

"Los Alamos has been doing epidemiological modeling for decades, starting with HIV," says Paul Fenimore, EpiGrid project leader. "It's a capability we were very wise to develop." For the sudden emergence of COVID-19, Fenimore and his colleagues strive to make EpiGrid as reliable as it already is for infections like plague or cholera. So they work the problem in both directions: in January, they forecast February, and in February, they retroactively assess what did and didn't work in the forecast in January.

Another key model, EpiCast, has similarly deep roots—but from a completely different kind of soil. Rather than being built from the ground up for epidemiology, EpiCast was adapted from an earlier materials-science model designed to support nuclear weapons technology. Just as individual atoms contribute to the nature of a material, individual infected people contribute to the progression of an epidemic, and the model is structured to treat each element (atoms or people) in an agent-based fashion,

tracking its influence and that of its neighbors to their ultimate global effects. Whereas EpiGrid typically covers large regions in aggregate (e.g., the eastern half of the country) with medium-grain resolution, EpiCast resolves down to the census-tract level, consisting of only about 2000 individuals, capturing their contact networks and daily travels, as well as any pandemic-related policy restrictions on either.

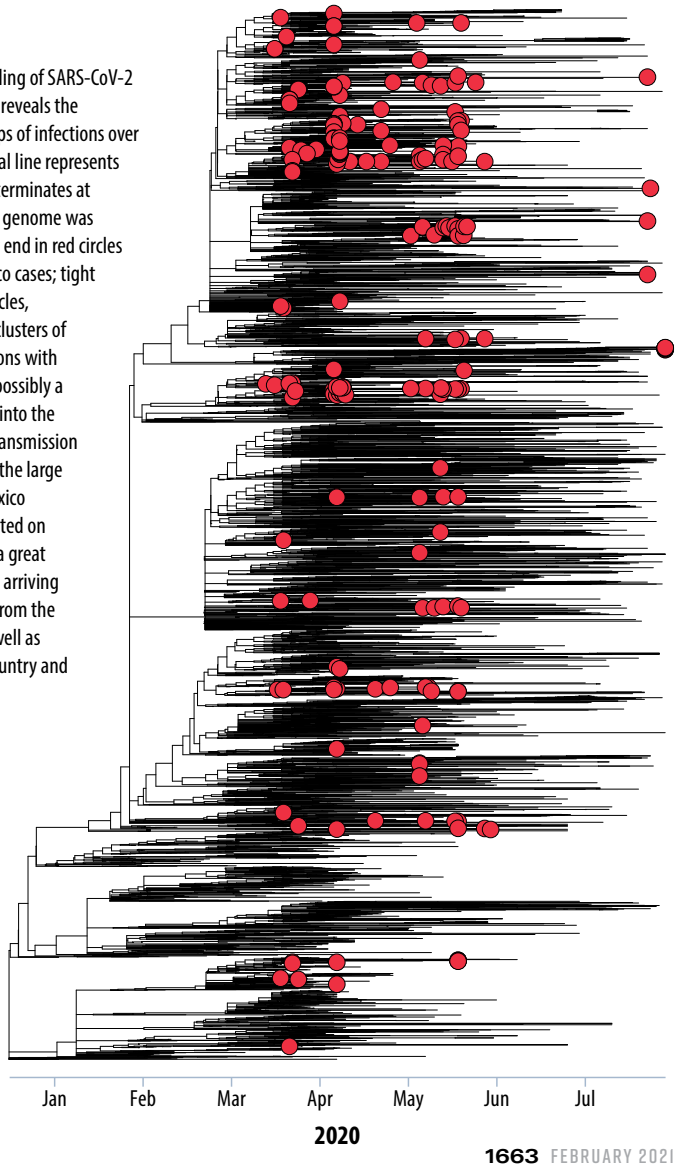
Not surprisingly, operating a model with such resolution requires a powerful computer. While EpiGrid can run on a laptop, EpiCast requires a supercomputer—and Los Alamos has several. In fact, Los Alamos has long been a key player nationally in high-performance computing (HPC) across the board, always keeping up with cutting-edge hardware, expert personnel, and scientists studying both the complex systems that require HPC for their simulations (e.g., climate models) and the science of HPC itself (such as minimizing error rates and applying different algorithmic approaches). Los Alamos HPC capabilities are currently being shared across a broad consortium of national laboratories and government agencies, universities, and technology companies to make supercomputers—which are normally prohibitively expensive for smaller organizations—freely available to researchers working to combat the virus with computationally intensive tasks such as drug discovery.

With Los Alamos's own agent-based HPC pandemic model, the results are especially credible, since the "agents" are essentially actual Americans: EpiCast incorporates real census counts combined with accurate demographics, school and workforce participation, and public-transit commuter information, among other key parameters. In addition, a key differentiator between EpiCast and other similar efforts is its ability to categorize workers within different industry sectors. This feature proved critical in understanding and projecting the pandemic in the United States by taking into account the variability in work-from-home policies affecting different segments of the workforce. The effects of changing mitigation strategies or individual behaviors thus percolate through an uncommonly realistic representation of the American populace. It is here that Los Alamos scientists Tim Germann, Carrie Manore, and Sara Del Valle can model those difficult-to-model human behaviors and analyze which ones are most effective in slowing the pandemic. As a result, EpiCast has been able to meaningfully assess the impact of reopening schools and workplaces.

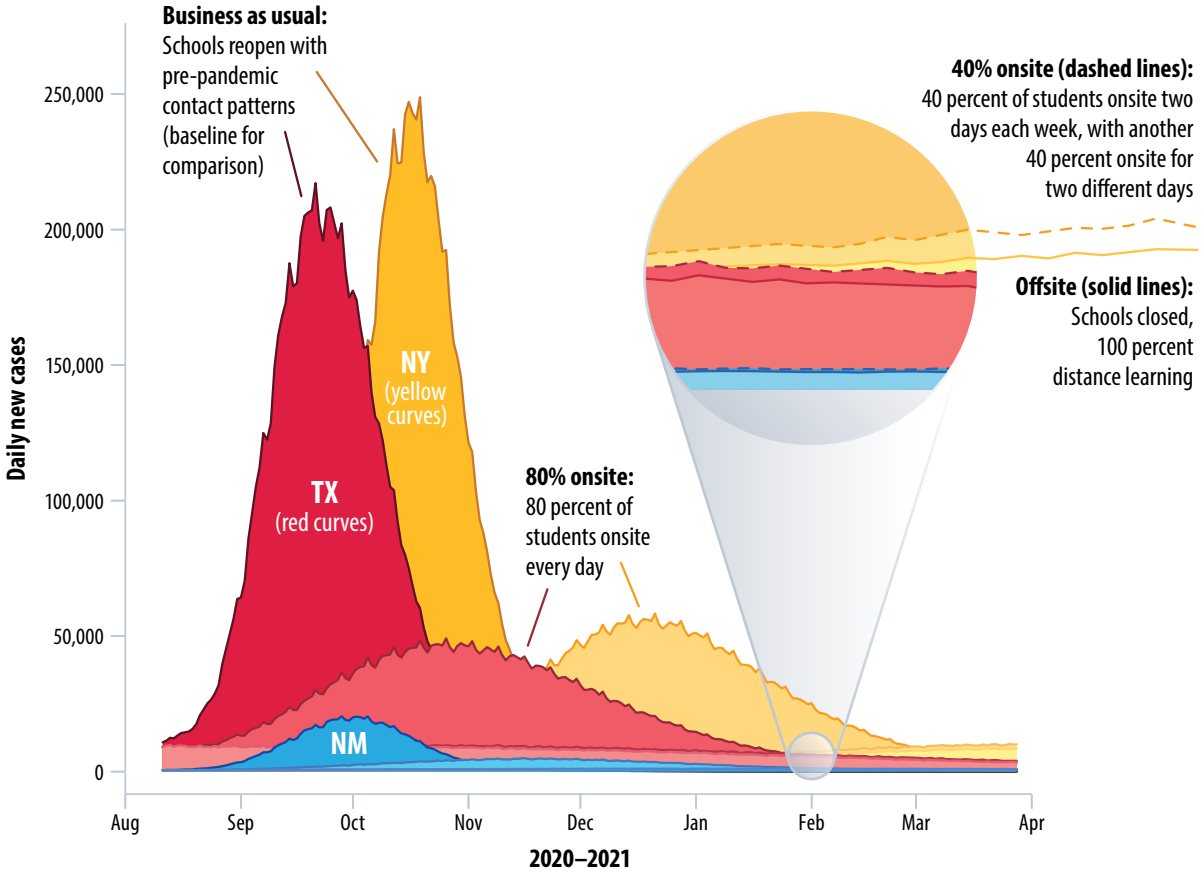
What does happen

Inferring the movement of the virus from epidemiological data, such as interviews with infected people to pinpoint where they have been and with whom they have had contact, results in an incomplete picture, making it difficult to calibrate models with real-world data. Los Alamos scientists Emma Goldberg, Ethan Romero-Severson, and Thomas Leitner are therefore tracking the movement of the virus with direct analyses of its genome as it migrates through the human population. Small, natural mutations are always happening to individual viral particles, and they happen at a fairly steady rate of approximately one or two nucleotides (basic elements of genetic code) every one or two weeks. That stream of inherited changes makes it possible to draw conclusions along the lines of whether *this* person could have acquired SARS-CoV-2 from *that* source (person, hospital, city, etc.) over *such and such* a timeframe when the viral genomes are so different.

By tracing what the mutations show about the relatedness of infections, i.e., the phylogenetics—a capability Los Alamos previously advanced to address the evolution of HIV infections—the scientists can help identify how and when the virus traveled from one region to another. This makes it possible to reliably tease apart whether a resurgence of cases in one area was caused by community spread within that area or by reinfection from the outside. The answer matters: if it's the former, then it might make sense to double down on isolation measures, such as closures and social distancing; if it's the latter, it might be more consequential to restrict interstate travel. In this way, real-world genomic data can be used to identify what happened in specific regions at specific times—and also validate (or contradict) models such as



Phylogenetic modeling of SARS-CoV-2 genome sequences reveals the relatedness of groups of infections over time. Each horizontal line represents a viral lineage and terminates at the time when that genome was sampled. Lines that end in red circles are from New Mexico cases; tight groupings of red circles, therefore, suggest clusters of New Mexico infections with a common source, possibly a single introduction into the state followed by transmission within it. However, the large number of New Mexico cases widely separated on this figure suggest a great many introductions arriving at different times (from the Mountain West as well as elsewhere in the country and the world).



Can schools be re-opened safely? Shown here are the projections of an EpiCast model from August 2020, presenting the anticipated number of new cases daily (vertical axis) versus time, assuming different approaches to school re-openings. Compared to a business-as-usual school reopening (tall peaks), reduced onsite learning significantly diminishes peak new cases—flattening the curve to reduce the peak burden on the healthcare system. Reducing to a plan with 40 percent of students onsite at one time (two cohorts, with two days per week for each) cuts new cases down to a rate much closer to that obtained by 100 percent remote learning. The model takes into account the initial conditions in each state (at the time the model was run) and the regional demographics, including how many people work in industries that are still operating onsite during the pandemic. This accounts for the state-by-state differences. As a result, Texas, for example, would see an earlier peak than most other states and New York a later one. New Mexico, home to Los Alamos, would peak in between.

EpiCast, allowing them to more accurately extrapolate and predict the direction of the pandemic across the country.

"Of course, we need up-to-date genome data to make up-to-date inferences," says Goldberg. "That's why we're coordinating with the University of New Mexico, TriCore Reference Laboratories, and the New Mexico Department of Health to continue to get viral genomes as more infections are confirmed in state." She and Romero-Severson are performing sophisticated statistical analyses to pull patterns from this in-state data, combined with other publicly available genomic data shared from across the globe. Such patterns reveal actionable characteristics of the movement of the virus—for example, which groups of cases trace back to a single introduction into New Mexico and how the number of such introductions is changing over time.

The epidemic curve is really a learning curve.

Meanwhile, Leitner is comparing current SARS-CoV-2 phylogenetics with those of other recent coronavirus outbreaks, including SARS-CoV and MERS-CoV, and with other types of resident coronavirus infections in animals, such as bats. In addition, a user-friendly web interface for genomic science, built by Los Alamos bioinformatics specialist Patrick Chain and his colleagues, is now being used to help automate the reconstruction of SARS-CoV-2 genomes for inclusion in phylogenetic trees and public genome repositories. The system analyzes the population of viral genomes found in a sample from a COVID-19 patient and identifies specific mutations and their prevalence. There is also a feature for evaluating how effective current high-quality viral-RNA-based COVID-19 diagnostic tests are at recognizing emerging genetic variants. And all of this work—phylogenetic analysis, pattern extraction, comparative studies, genome reconstruction, and diagnostic-test validation—capitalizes on Los Alamos computing technology and expertise.

In addition to geographic, phylogenetic, and behavioral aspects, a final key element of the Los Alamos modeling effort is systemic and capitalizes on a major research initiative from the previous decade. From 2003 to 2010, Los Alamos scientists modeled the nation's critical infrastructure—things like power, transportation, and, of particular relevance now, public health—to expose their interdependencies and learn how to maintain them in a crisis. When the COVID-19

pandemic struck, Los Alamos scientist Jeanne Fair [see *In Their Own Words* on page 2] and fellow researchers Rene LeClaire, and Lori Dauelsberg—all of whom were key players in the critical-infrastructure study—responded quickly to restore that capability and adapt it to the current pandemic.

As part of this process, they had to rework an earlier epidemiological model of an influenza pandemic scenario so that it would properly account for the very different scenario brought on by a coronavirus. The result, known as MEDIAN (Modeling Epidemics for Decision support with Infrastructure ANalysis), is a suite of system-dynamics models designed to identify the key drivers of the pandemic. It explores the large uncertainties pertaining to the disease itself—things like incubation period and mortality rates—together with the way society's infrastructure systems function to make things better or worse.

For example, one often hears about the danger of simply "overwhelming the healthcare system," but the healthcare system is a complicated animal. People are routed among home care, physicians' offices, hospitals, intensive-care units, emergency rooms, and long-term care facilities. Medical services can include multiple types of COVID-19 testing and treatment, and the selection of services could have significant impacts on the trajectory of the pandemic. The MEDIAN team is looking at which knobs to turn to most affect the outcome, and it has been tasked in particular with understanding the uncertainties associated with testing and diagnostics to help identify an optimal testing strategy.

What should happen

COVID-19 is a killer, and Los Alamos is doing everything it can to provide life-saving scientific guidance for policymakers. The four-lab collaboration between Los Alamos, Argonne, Sandia, and Oak Ridge national laboratories has been fruitful in this regard. Just as Los Alamos is particularly well positioned to provide expansive modeling and diagnostics, partner labs have their own specialties that collectively contribute to overall situational awareness. Ben McMahon, who continues to learn everything he can to help accelerate the nation's learning curve, is paying close attention.

"Weekly reports between partner labs reveal an ever-expanding, ever-sharpening picture," says McMahon, "but they also deliver a healthy dose of humility. They increase what we know and refocus our attention on everything we don't."

Within Los Alamos's home state, this knowledge—incomplete though it may be—is making a big difference. Throughout the crisis, Laboratory experts have been in regular contact with New Mexico state officials, hospital representatives, mental health specialists, regional economists, and other policy professionals. Typically, two or three conference calls per week allow vital information to be shared as soon as it is discovered. Additionally, state officials can get scientific evaluations from Los Alamos on the questions that arise day to day, such as whether a new cluster of cases is likely to represent a "real" problem or a statistical blip, or how best to distribute the available COVID-19 tests. Major policy announcements or changes are made only after extensive discussions with a diverse set of experts, including Los Alamos scientists from many disciplines.

"Los Alamos serves the entire nation with its resources, capabilities, and expertise, but the partnership between Los Alamos and the state of New Mexico has been extraordinarily productive for everyone involved as well," says Kirsten McCabe of the Lab's National Security and Defense Program Office. "We are fortunate to be able to interact with the state government and Presbyterian Healthcare Services and to have a proactive governor making informed decisions to manage the crisis. Critical information flows freely in both directions."

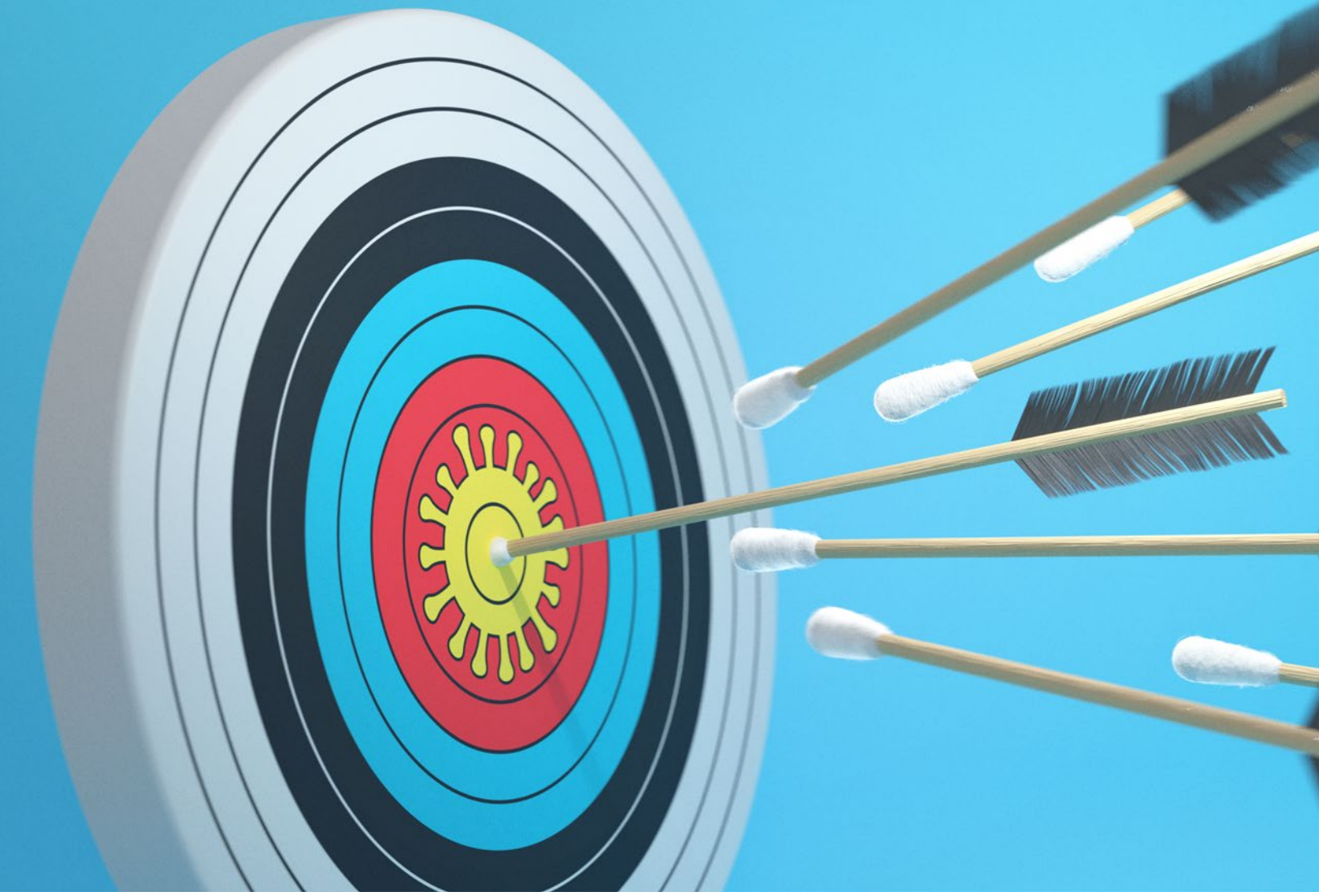
Models have to accept flawed data and generate the best possible prediction anyway.

Exponential change goes in both directions, too. If one infected person infects five more, then 25, then 125, the cases will skyrocket. But if one infected person infects one-tenth as many—0.5 on average, say—then exponential growth reverses and becomes exponential decay: 20 cases become ten, ten become five, and any new flare-up dwindles away. If model-informed policies can put the population firmly in the exponential-decay domain, then careful, controlled attempts to restore particular elements of normal life can be attempted relatively safely. With great vigilance to rapidly isolate and contact trace new cases as they appear, the prevailing condition of exponential decay can be relied upon to do its thing.

"The math works with us or against us," says McMahon, "but it's a very fine line. It all hinges on having extremely accurate models and acting on the best possible information."

Like many of his pandemic-modeling colleagues at Los Alamos and around the world, McMahon feels frazzled. But there is no rest. Until scientists know much more about this virus, the weight of the world will continue to hang on a select few, including healthcare workers, elected leaders, and yes, modelers, who continuously reshape shifting uncertainties into the most likely truths. They are, after all, the ones specifically entrusted with advancing our learning curve. LDRD

—Craig Tyler



DETECTION

ON-TARGET TESTING

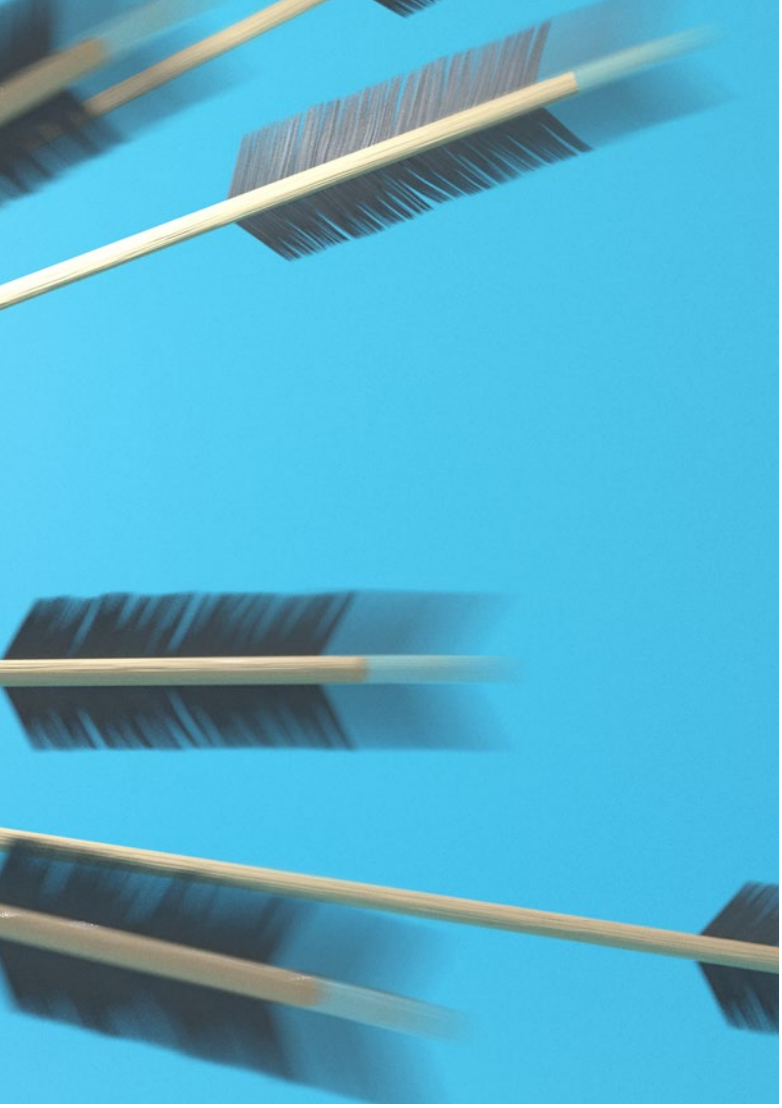
Los Alamos's multifaceted response includes running an in-house testing center and developing the next generation of COVID-19 tests.

"DO I HAVE COVID?" This time last year, the question was not in our collective vocabulary. But today, symptoms such as a fever, cough, or headache could prompt a person to wonder: is it COVID-19 or just a cold? Or maybe the flu? These days, this is a very important distinction. Furthermore, based on evidence that people can spread the SARS-CoV-2 virus without showing any symptoms whatsoever, "Am I infectious?" is another important new question to ponder.

Accurate, rapid diagnostic testing prevents disease spread by identifying infectious people who should self-isolate. Testing also helps scientists understand key characteristics of a disease outbreak such as infectivity rate and patterns of spread. There are many challenges, however, to implementing widespread testing for a novel virus such as SARS-CoV-2, and throughout the pandemic, testing availability and reliability have been variable across the United States and around the world. This makes it difficult to contain the virus because when people aren't aware that they are infected, they are more likely to spread the disease.

Current COVID-19 tests fall into two categories. Lab-based tests that detect the RNA genome of the virus are the most reliable, but they are expensive and require skilled technicians and equipment that are limited in availability. Rapid tests that detect viral proteins are easier to administer and can give results in as little as 15 minutes; however, many are far less accurate than RNA tests. Further, both types of tests could show false negatives if used too early or too late during an infection, when there are fewer virus particles to detect.

Since the beginning of the pandemic, Los Alamos has been addressing these challenges in multiple ways. The Laboratory quickly established a new onsite testing lab and also contributed



valuable information to state and federal governments about test efficacy. In addition, Los Alamos programs—built on long-standing capabilities in genomics and protein design—are refining existing tests and also creating new approaches that could improve the future of testing overall.

Testing, testing everywhere

When the novel coronavirus SARS-CoV-2 emerged in late 2019, scientists rapidly determined its full genomic sequence in order to identify exactly what was making people sick. This sequence of about 30,000 ribonucleic acid (RNA) bases is effectively the viral blueprint: it contains all the information necessary to build the virus. The sequence also shows how SARS-CoV-2 is different from other coronaviruses, especially the closely related 2002 SARS virus (SARS-CoV). Even small variations in genetic sequence can translate into major differences in how the virus behaves and why it caused a global pandemic.

Diagnostic tests, however, do not require a full genomic sequence to be determined from each person's nasopharyngeal swab. Instead, because the genome sequence is known, scientists can identify unique signature regions of the RNA to target in a process called reverse transcriptase polymerase chain reaction (RT-PCR). RT-PCR is considered the gold standard for diagnosing SARS-CoV-2.

Clinical labs often conduct RT-PCR tests, or assays, for diagnostics, but this technology is also widely used in biological research. The presence of PCR machines and skilled personnel in many academic institutions and the DOE national labs meant that additional COVID-19 testing capacity could be added quickly. In April 2020, Los Alamos managers and scientists created an in-house COVID-19 Testing Lab to test Los Alamos staff and to provide support for the New Mexico Department of Health, if needed.

"We were happy to provide this service to the institution," says Los Alamos Bioscience Deputy Division Leader Alina Deshpande, who manages the COVID-19 Testing Lab. "In fact, our Biological Agent Testing Laboratory (BATL) was already accredited for test evaluation, so it was relatively easy to prepare for the additional certifications."

Since 2009, the BATL has been evaluating tests based on nucleic acids for various government programs. In other words, Los Alamos would study assays to determine if they accurately detect the pathogen they claim to detect. To do this, the BATL team obtained accreditation from the International Standards Organization, demonstrating its adherence to regimented operations and protocols, such as unidirectional workflows, segregation of workspaces, and strict chains of custody—all to reduce contamination risk.

To process clinical human samples, Deshpande and her team applied for Clinical Laboratory Improvement Amendments (CLIA) registration, which is required for all diagnostic labs. This additional certification required a few steps, such as customizing the information management system and proficiency testing for all staff with blinded samples.

Medical technicians at Los Alamos's Occupational Medicine group take nasopharyngeal swabs from patients, put them in preservative reagents called viral transport media, and send them to the COVID-19 Testing Lab. There, RT-PCR is done to confirm the presence or absence of SARS-CoV-2 RNA in the sample. Since April, a cohort of about 20 staff members in the Bioscience Division have balanced their normal research priorities alongside taking shifts in the Testing Lab.

The Los Alamos Testing Lab, like others, uses COVID-19 testing kits, reagents, and procedures authorized by the Food and Drug Administration (FDA). The FDA grants Emergency Use Authorizations for specific products and methods as guidance to all clinical labs about what kits are acceptable during the pandemic. To help with this, the FDA and the Centers for Disease Control (CDC) call upon Los Alamos and other national labs to provide independent evaluation of various COVID-19 testing components.

"We are providing data to the FDA and CDC on the effectiveness of different tests and methods to help them make COVID-19 testing decisions at the national level," explains Bioscience Division Leader Elizabeth Hong-Geller. Hong-Geller is the Los Alamos lead for the R&D lab testing working group within the National Virtual Biotechnology Laboratory, which was established to coordinate COVID-19 research among the Department of Energy's national laboratory complex. "We have investigated such questions as the stability of nasopharyngeal swabs at different storage temperatures and the reliability of different viral transport media to ensure that RNA on the swabs doesn't degrade during transport from the clinic to the testing lab."

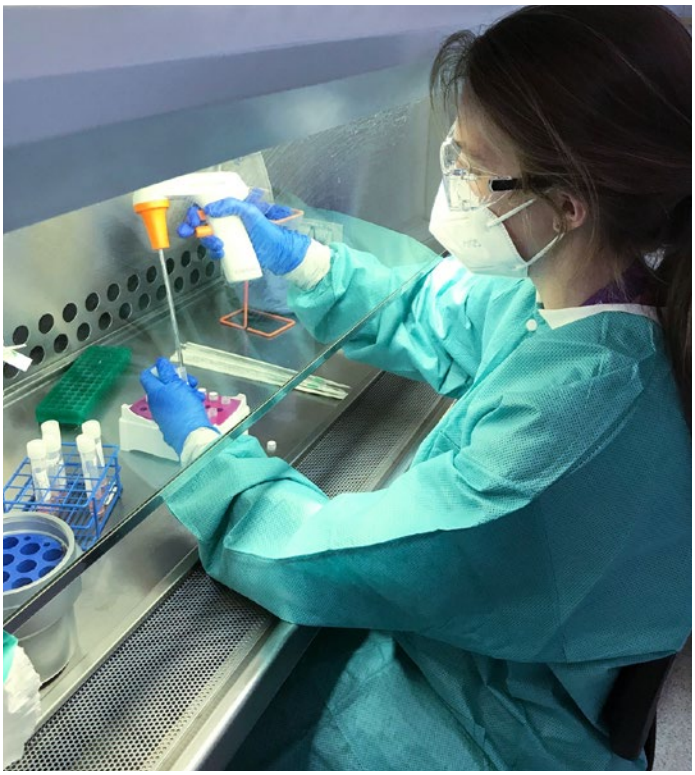
Start with the sequence

The first diagnostic tests for COVID-19 were based on the full genomic sequences of SARS-CoV-2 that were available in early 2020. Since that time, scientists around the world have continued to assemble thousands more SARS-CoV-2 sequences for comparison. Most genomes are deposited into online databases such as the National Institute of Health's GenBank and the Global Initiative on Sharing All Influenza Database (GISAID), which expanded to include SARS-CoV-2. As of today, there are nearly 200,000 genomes available to scientists, which is vitally important for an effective pandemic response.

In March 2020, Los Alamos bioinformaticist Patrick Chain and his colleagues quickly adapted their award-winning open source software EDGE (Empowering the Development of Genomics Expertise) to help others contribute SARS-CoV-2 genomes to these databases. The EDGE platform provides automated workflows to help users assemble raw genomic data into high-quality complete genomes that are more useful for research. So far, users in more than 50 countries have used COVID-EDGE for their SARS-CoV-2 genomes.

The sequences in these databases help researchers track and study mutations in the SARS-CoV-2 genome to better understand the virus's phylogeny, or family tree. Because the genetic sequence determines the virus's structure, the locations of mutation within the genome have important implications. Multiple teams at Los Alamos are evaluating sequence data to track mutations for developing therapeutics and vaccines. Chain's team is studying current PCR-based tests and has developed an assay-validation tool to determine if tests continue to accurately detect SARS-CoV-2 as its genome mutates.

"This validation can help identify when mutations may interfere with diagnostic tests or even therapeutics. If mutations arise in the RNA regions targeted by an assay, and if these mutations are maintained in the circulating population, it could result in assays no longer being as effective," says Chain. "Our tools are constantly and automatically being updated to identify where on the planet mutations are arising that make assays more likely to fail."



Los Alamos technician Julie Strickland in the Los Alamos COVID-19 Testing Lab.
CREDIT: Omar Ishak/LANL

Accurate testing prevents disease spread by identifying infectious people who should self-isolate.

Primers, probes, and PCR

The RT-PCR assay is based on some key processes in living cells: complementary binding and nucleic-acid replication. When cells divide to make more cells, they replicate their DNA using enzymes called polymerases. The double-stranded DNA separates into two template strands where the bases are exposed to allow a new copy to be made. The polymerases create a copy strand by assembling new pieces of DNA according to complementary binding with the template: this dictates that the base adenine (A) binds to thymine (T), and guanine (G) binds to cytosine (C). (When an organism has RNA instead of DNA, as in the case of SARS-CoV-2, the enzyme reverse transcriptase is used to make a DNA copy via complementary binding to the RNA template.)

In 1985, scientists developed PCR as a way to mimic this process in a lab setting in order to copy small, undetectable amounts of DNA many times over, amplifying it to a point where it becomes detectable. PCR can also be used as a diagnostic assay by only amplifying signature regions that identify a pathogen. This requires lab-made DNA primers, which determine where along the organism's DNA strand the replication process will begin, and probes (also made of DNA), which produce a signal—such as a distinctive fluorescent glow—to indicate when amplification of

the target has happened. For this reason, the design of primers and probes that reliably recognize SARS-CoV-2 is critical to the effectiveness of the assay.

Los Alamos has multiple projects underway that include advanced primer and probe design for SARS-CoV-2. Overall, making primers and probes is a mathematical optimization problem that must take into account all of the known viral genomic diversity—including any mutations—and also satisfy other criteria for the assay to work.

"You need primers and probes that are simultaneously conserved in and unique to the pathogen of interest," says Jason Gans, a Los Alamos computational biologist. Genes that encode for viral spike proteins are some of the places where mutations have occurred that distinguish SARS-CoV-2 from other closely related viruses. Additional criteria for good primers and probes address size and composition. A good size for probes is about 30 bases; they should include significant amounts of G-C pairs for thermodynamic stability; and they should not inadvertently bind to each other, only to the template.

In one Los Alamos project, Gans and his colleagues are optimizing probes for multiplex assays that detect multiple pathogens at once. This would be especially useful during flu season when there are many viruses in circulation. Gans and his colleagues optimized primers and probes for each target that will not bind to each other or cross-compete for binding sites when they are used at the same time. Furthermore, each probe requires a fluorescent tag, so the team is working to reduce background fluorescence from the multiple probes to ensure a clear signal. So far, Gans says they have designed an assay that simultaneously detects three pathogens and are now aiming for up to sixteen.

Gans is also working with another, larger team at Los Alamos to develop probes for a more portable kind of diagnostic test—one that would still be RNA-based but would eliminate the need for a PCR machine. Biochemist Jessica Kubicek-Sutherland and theoretical biologist Karina Yusim lead a multidisciplinary team that has adapted a project they already had underway—called Fast Evaluation of Variable Emerging Risks (FEVER). The FEVER project first focused on designing probes and instrumentation to support both diagnostics and global surveillance of influenza viruses. When SARS-CoV-2 emerged, the team began to use their FEVER strategy to optimize new coronavirus probes.

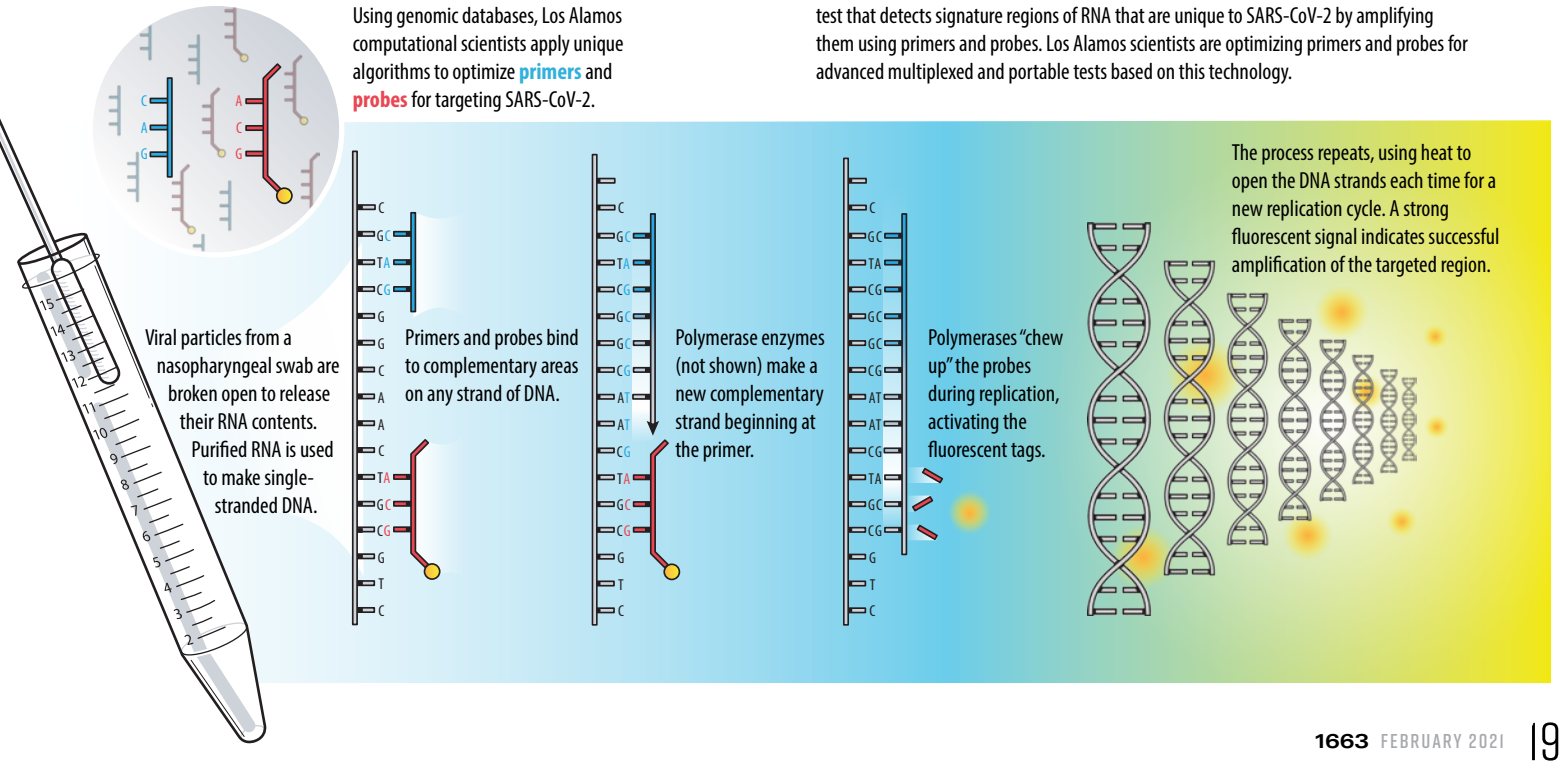
"We developed two probes that react with all SARS-like viruses, including SARS-CoV-2," says Kubicek-Sutherland. These probes are useful for surveilling genomes sampled from various animals to look for newly emerging coronaviruses. "We also developed two probes that only detect SARS-CoV-2 and do not react with any other SARS-like viruses," she continues. Together, these four coronavirus probes comprise an assay that can be used for both specific SARS-CoV-2 diagnostics and universal coronavirus surveillance, which is important for identifying the next potential pandemic threat.

Primers and probes that reliably recognize SARS-CoV-2 are critical to the effectiveness of RT-PCR tests.

The team is currently validating its probes in clinical samples, and it is also examining probe sensitivity using saliva, which would be less invasive than nasopharyngeal swabs and does not require a trained technician. The use of saliva is one important aspect of a more portable diagnostic; the other is simplified instrumentation.

The ultimate goal is to adapt these probes to a format that does not require PCR amplification. This would significantly

Reverse transcriptase polymerase chain reaction (RT-PCR) is a common lab-based diagnostic test that detects signature regions of RNA that are unique to SARS-CoV-2 by amplifying them using primers and probes. Los Alamos scientists are optimizing primers and probes for advanced multiplexed and portable tests based on this technology.



reduce the amount of time and cost it takes to get a test result. Kubicek-Sutherland is part of a Los Alamos team led by Harshini Mukundan that has spent years designing a waveguide biosensor that uses a laser to identify molecules for pathogen detection and is currently adapting the biosensor to be more portable. The team envisions a future with miniature versions of this technology—paired with FEVER probes—which together could eliminate the need for lengthy lab tests by making RNA detection more accessible.

Take-home testing

Waiting several days for a COVID-19 test result makes it difficult to contain the pandemic. For this reason, scientists worldwide have developed—and are improving—rapid tests, many of which use antibodies to detect viral proteins. Suitable inexpensive antibody-based technologies, called immunoassays, have existed for many years. The key ingredient to making immunoassays into a reliable COVID-19 test is highly selective antibodies that only target SARS-CoV-2 and strongly bind to it.

Antibodies are specialized proteins that "recognize" various molecules called antigens—most notably, helping immune systems "remember" prior infections. Antibodies do this through a lock-and-key interaction: a specific area on the antibody recognizes and binds to a specific antigen (often on the surface of a virus or bacterium). People who are battling a COVID-19 infection will develop antibodies that recognize SARS-CoV-2 antigens, which is why COVID-19 antibody tests can indicate that a person has already had the disease.

Diagnostic tests can also use this antibody-antigen relationship to detect active, current infections. However, although the antibodies produced by a human during an infection are effective enough to summon an immune response, they are not particularly reliable for diagnostics because one antibody may recognize multiple organisms. If the antibodies in a test recognize many similar coronaviruses, the test could give a false positive result. On the other hand, if the antibody doesn't bind to enough virus particles, it could give a false negative.

TRACKING THE INVISIBLE THREAT

Detecting virus particles once they leave their human hosts is the focus of another National Virtual Biotechnology Laboratory project—Viral Fate and Transport—which is led by Pacific Northwest National Laboratory and Lawrence Berkeley National Laboratory. Large droplets produced from coughing or sneezing are known to fall out of the air quickly. However, smaller ones (less than 100 microns, or millionths of a meter) that are produced when people speak, sing, or shout can stay aloft in poorly ventilated spaces. Determining the impact of these small airborne droplets, called aerosols, is of utmost importance for keeping people safe.

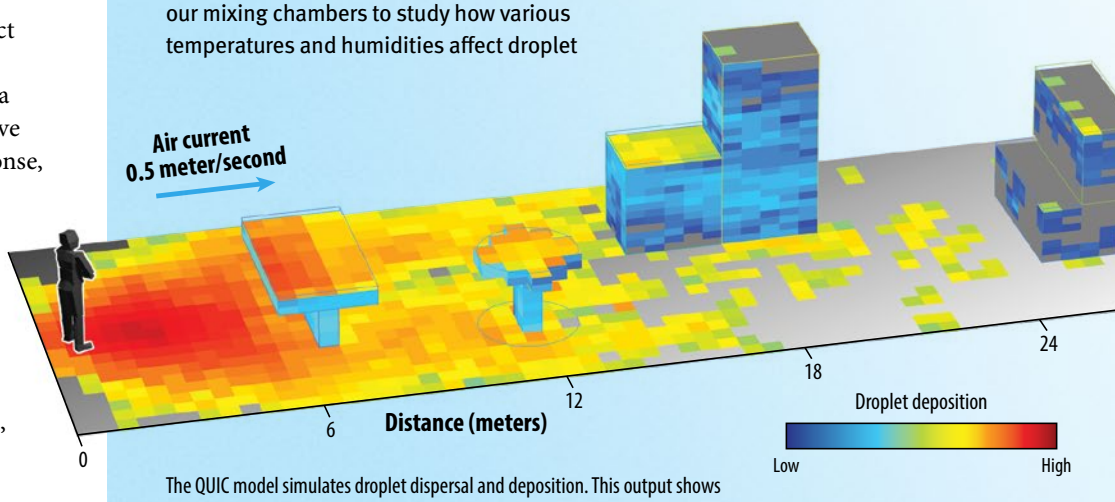
As part of this large project, Los Alamos atmospheric chemist and aerosol specialist Allison Aiken is contributing her team's expertise and instrumentation at the Los Alamos Center for Aerosol Forensics and Experiments (CAFE). Aiken explains that more experimental data are needed to understand aerosols under different environmental conditions and more realistic circumstances. For example, many previous experiments used simple saline solutions to simulate SARS-CoV-2-containing droplets, which are in reality more complex.

"Our team is investigating other surrogates that include proteins and organics in water droplets, in addition to saline, that can result in phase separations under different conditions," says Aiken. "Using these surrogate liquids, we can create a burst of different-sized droplets in our mixing chambers to study how various temperatures and humidities affect droplet evaporation. This is important because viral fate under low humidity is currently unknown for SARS-CoV-2."

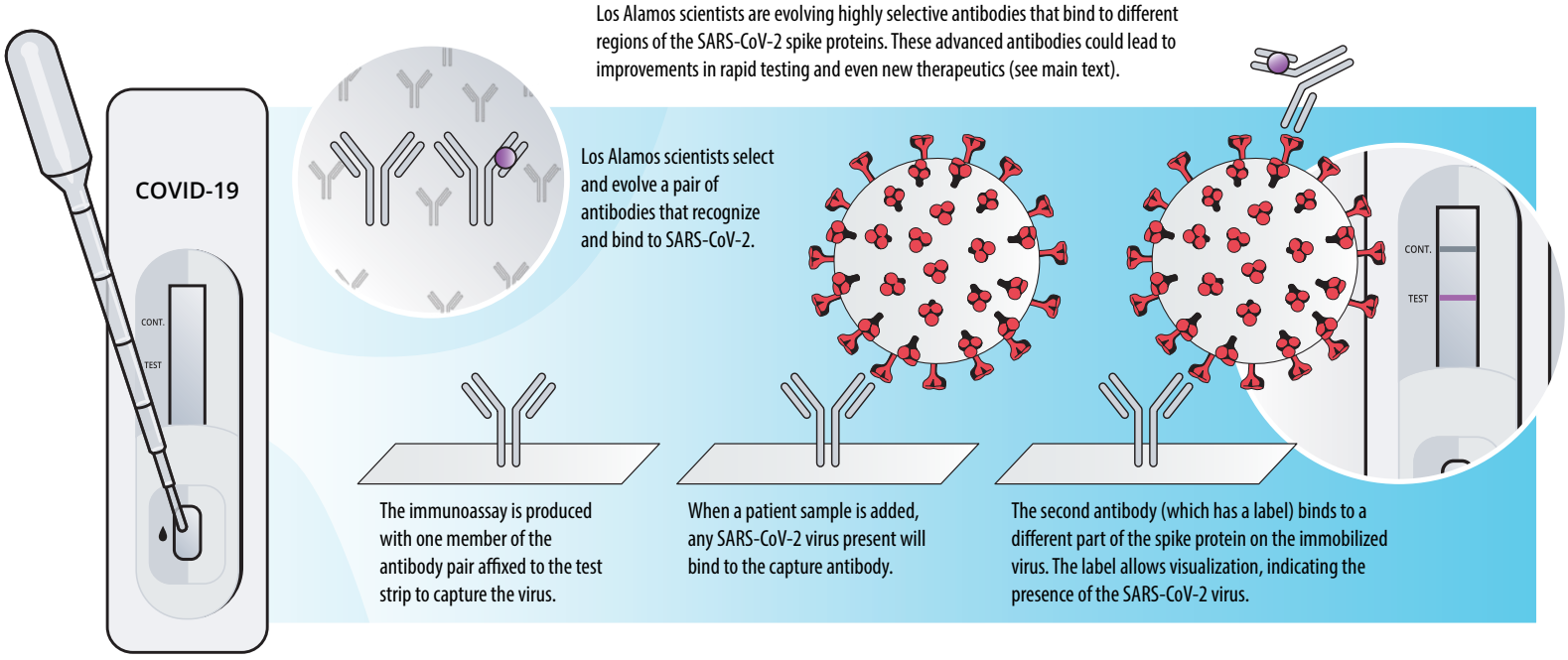
Using Aiken's experimental data, Los Alamos atmospheric scientist Michael Brown is using the Quick Urban and Industrial Complex (QUIC) droplet dispersion model to simulate how these various-sized droplets distribute in realistic spaces such as a restaurant courtyard.

"We can change the shape and geometry of the courtyard, adjust spacing of tables and furniture, add or remove overhead canopies, or modify the height of the walls to determine how to make it safer," says Brown. "We can even add potted bushes and trees to see how they modify the airflow and potentially filter out and catch the virus-filled droplets."

The Fate and Transport collaboration is also working on other fronts, such as detecting SARS-CoV-2 in wastewater. Wastewater monitoring could be used as an early warning system for future outbreaks—and sequencing wastewater-collected genomes is key. Los Alamos's genomic scientist Armand Dichosa is contributing to this effort by collaborating with wastewater plants on standardizing sample collection and preparation to get the most useful sequence data. Ultimately, by further understanding the fate and transport of SARS-CoV-2 in multiple environments, Los Alamos scientists hope to provide data to support improved public health guidelines.



The QUIC model simulates droplet dispersal and deposition. This output shows that a light breeze can transport evaporating 100-micron droplets more than 18 meters away from the infected individual and deposit them on various surfaces.



Protein-based immunoassays use antibodies to capture and detect viral particles. Los Alamos scientists are evolving highly selective antibodies that bind to different regions of the SARS-CoV-2 spike proteins. These advanced antibodies could lead to improvements in rapid testing and even new therapeutics (see main text).

Los Alamos biologists Geoff Waldo, Mietta Lillo, Nileena Velappan, Ramesh Jha, and Hau Nguyen, who comprise the Los Alamos Affinity Reagent Team (LA-ART), are using their long-standing capability to create pairs of highly specific antibodies that work in concert to target SARS-CoV-2 exclusively. The goal is to find two antibodies that will not compete for the same binding site on a target antigen so they can be used in an immunoassay where one antibody captures the virus and the second one creates a detectable signal. Other immunoassays use this approach, but often the target antigen is the SARS-CoV-2 nucleocapsid (a structural protein associated with the viral RNA); however, the Los Alamos team has chosen a different antigen.

"Our target antigen is a component of the SARS-CoV-2 spike protein called the receptor binding domain, or RBD," says Lillo. "The RBD is a key part of the spike protein that helps it attach to ACE2 receptors in the human body during infection. RBD is also the part of the spike protein where SARS-CoV-2 differs the most from the 2002 SARS virus."

The LA-ART scientists' approach uses microorganisms to produce and display human antibodies on their surfaces. These antibodies have the potential to recognize multiple coronaviruses, but the team is able to experimentally focus only on the antibodies that bind most selectively to the SARS-CoV-2 RBD target. The scientists select the microorganisms whose displayed antibodies bind to RBD and allow those microorganisms to reproduce. They repeat this process through multiple generations, evolving the microorganisms to produce antibodies with higher specificity, affinity, and robustness.

Using this technique, the LA-ART team identified 19 antibodies that selectively target the RBD of SARS-CoV-2. The team demonstrated that four of the antibodies, used as pairs, can detect small amounts of spike protein and viral particles at the average concentration found in clinical samples. Furthermore, those same four antibodies were shown to compete with the human ACE2 receptor in binding viral RBD: if the RBD has an antibody attached, it can't bind to the ACE2 and can't cause infection. This means the LA-ART antibodies have the potential to be used as therapeutics. This type of therapy would be similar to the antibody cocktail given to the president when he was hospitalized for COVID-19.

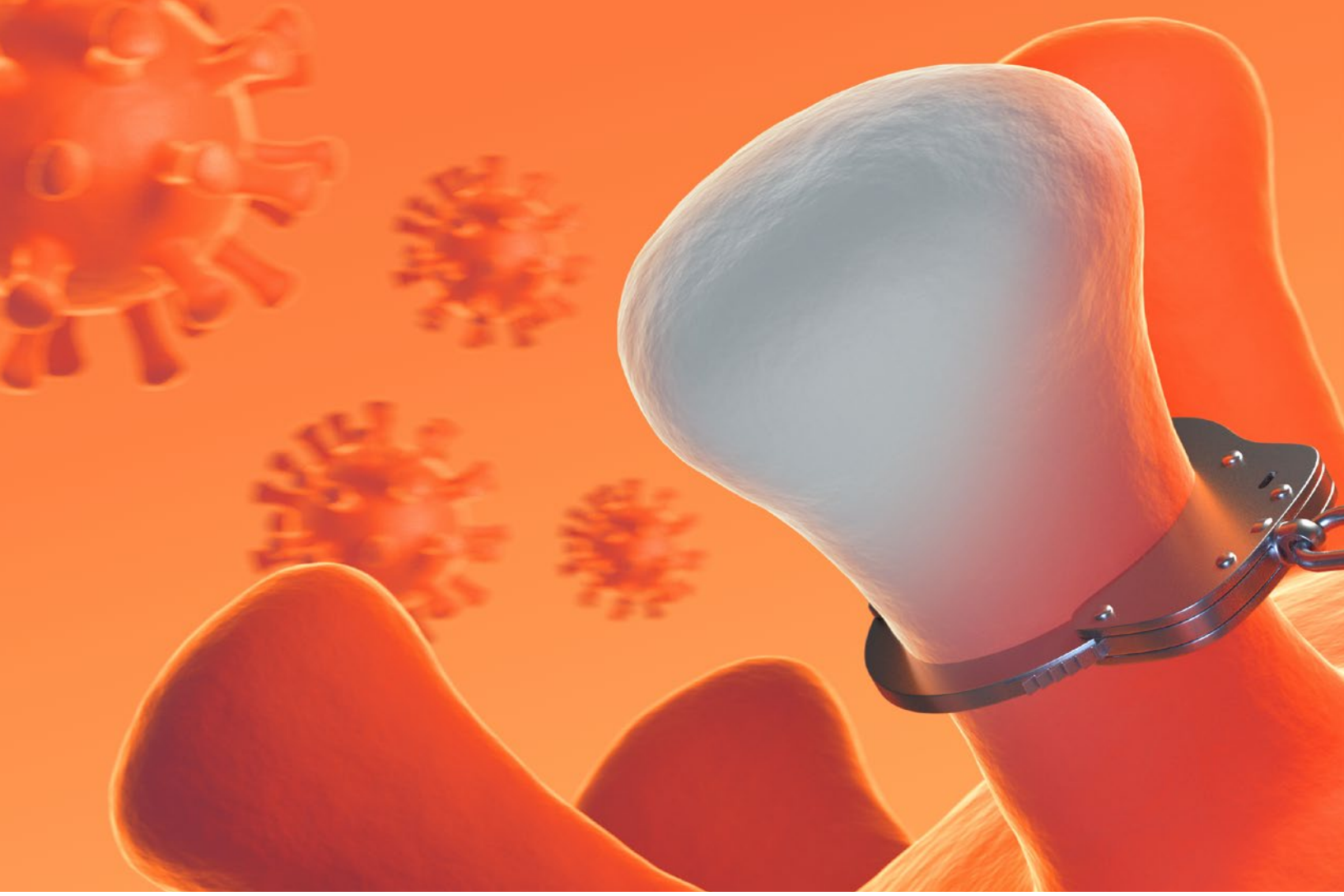
Reliable rapid tests can be developed using highly selective antibodies.

Furthermore, by isolating the microorganisms that produce the best-binding antibodies, the team is also able to obtain the genetic code for those antibodies, which will allow the scientists to improve the antibody-antigen interaction through modeling. "Using protein-modeling software, we are able to improve the affinity and change the binding specificity of some of the selected antibodies," says Los Alamos computational biologist Ramesh Jha. Jha and others also study the molecular dynamics of the protein-protein interactions, which helps them design other novel affinity molecules, similar to antibodies, from scratch.

The invisible enemy

The virus is everywhere, yet it is invisible. It has infected millions, killed hundreds of thousands of Americans, and left others with very few symptoms—and testing is the only way to find it. The nation and the world have come together to tackle this problem. Using research-laboratory assets and a wealth of sequence data, testing facilities and kits were quickly established to diagnose as many people as possible. But the pandemic is still growing, and for as long as people are getting sick, testing will be a major part of trying to contain it. By applying robust scientific approaches, Los Alamos scientists are designing the next generation of tests to help us control this invisible threat. LDRD

—Rebecca McDonald



MEDICAL COUNTERMEASURES

CATCHING THE CORONAVIRUS

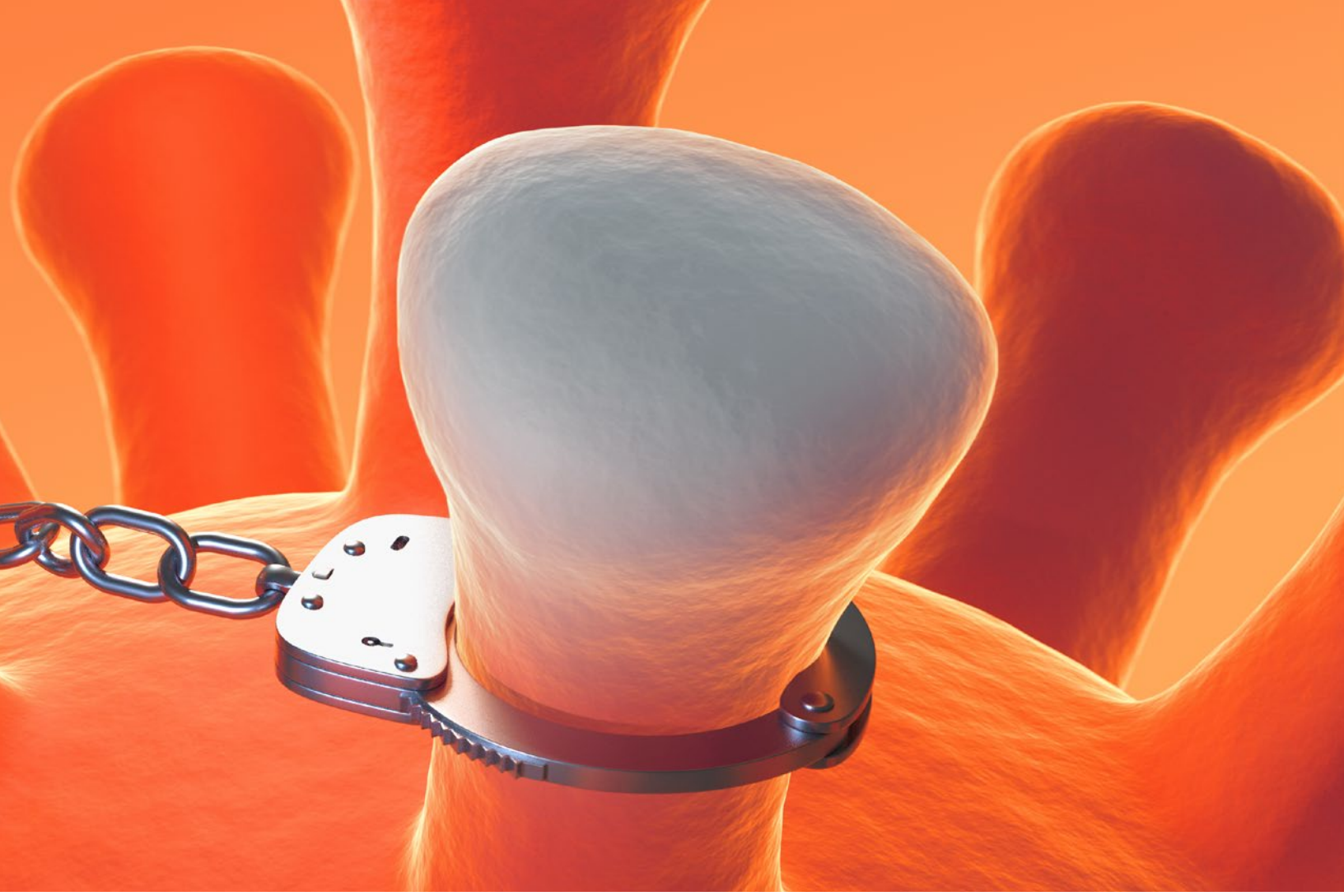
Laboratory scientists are coming at the coronavirus from all angles, exploring a multitude of ways to prevent infection and treat disease.

THE WORD VACCINE COMES FROM *VACCA*, the Latin word for cow. When 18th century English scientist Edward Jenner began inoculating people against the highly lethal smallpox virus (*Variola major*) by using a fairly benign related virus, cowpox virus (at the time called *Variola vaccinae*, or "smallpox of the cow"), he called the procedure "vaccination," derived from *vaccinae*, derived from *vacca*. Jenner had observed that milk maids who had cowpox seemed to be protected from smallpox. But infection by one virus providing immunity against subsequent infection by another, a phenomenon known as cross-protection, doesn't always succeed.

Most humans have been infected with one or more cold-causing coronaviruses in the past. But unlike the cowpox-smallpox scenario, prior infection by a less pathogenic coronavirus does not seem to protect against its bigger, badder cousin, SARS-CoV-2. Because there is no preexisting immunity to this virus in the human population, it falls to medical science to come up with ways to prevent people from becoming infected by SARS-CoV-2 and treat people who develop the disease it can cause, known as COVID-19.

To coordinate effort and address key challenges in responding to the pandemic, the Department of Energy established a consortium of national laboratories called the National Virtual Biotechnology Laboratory, or NVBL. One research area within the NVBL project called Molecular Design to Inform COVID-19 Medical Therapeutics is led by Los Alamos Office of Science Program Director and biochemist Srinivas Iyer.

"We are studying vaccine strategy, virus-host interactions, and small-molecule therapeutics," says Iyer. "By integrating structural biology, computation, modeling, machine learning, and other national-laboratory capabilities, the NVBL will accelerate the development of vaccines and therapeutics against COVID-19."



Preventing infection

In February of 2020, as SARS-CoV-2 was beginning to spread from its early enclaves to the rest of the world, Los Alamos theoretical biologist Bette Korber began to track the slowly accumulating genetic changes in the virus. Her goal was to help experimentalists identify different versions of the virus containing certain genetic changes that could impact vaccine efficacy. As a theorist, Korber studies the biology and evolution of highly pathogenic viruses, such as the Human Immunodeficiency Virus (HIV), Ebola, and Hepatitis C, and human immune responses to them. By early April, Korber saw something that, to her expert eye, looked like a pattern that was unlikely to be due to random mutation.

Coronaviruses are so named for the protuberant spike proteins that stick out from their surface, creating a halo, or *corona*—Latin for crown—around the particle. These spike proteins help the virus enter a human cell, so they are an obvious subject of scientific scrutiny. The spike protein for SARS-CoV-2 is formed from a string of 1,273 amino acids, and it was here that Korber saw the pattern: in the virus's original form, the 614th amino acid is aspartic acid (denoted by the chemical symbol "D"), but an increasing number of samples from disparate geographic locations had a glycine ("G") at that location. The replacement of D with G at location 614, or "D614G" in genomics nomenclature, began appearing in viral gene sequences in January and by June was found in nearly all new samples.

Korber and her colleagues, Will Fischer, Hyejin Yoon, James Theiler, Brian Foley, Nick Hengartner, and Werner Abfalterer, developed a bioinformatics pipeline to analyze SARS-CoV-2 gene sequence data from GISAID, the Global Initiative on Sharing All Influenza Database that was developed for influenza but is now the central coronavirus database as well. By studying GISAID coronavirus sequence data from around the world, the team looked for variants that were repetitively increasing in frequency. Their statistical analyses of global patterns showed that the D614G substitution was under positive selection; in other words, something other than mere chance was making the G-form supplant the D-form again and again.

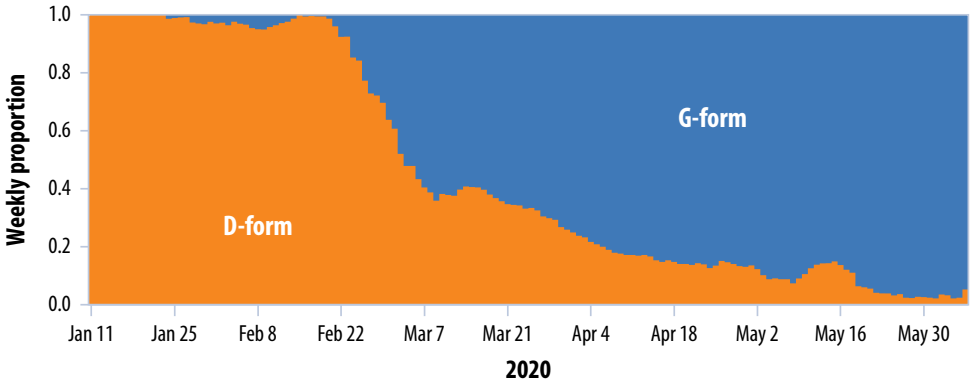
"The viruses carrying D614G were rapidly becoming the globally dominant form, and it was important to understand why," says Korber. "One possibility was that it was more infectious. Another possibility was that the substitution affected the human immune response to the virus." Most vaccine efforts were based on the ancestral D-form, but as the G-form was becoming dominant, it was important to ensure that a vaccine would be effective against that form as well.

The Los Alamos team, with collaborators from Duke University, the La Jolla Institute of Immunology, and Sheffield, England, compared G-form and D-form and found some

important differences. First, the G-form appears to replicate more readily in the upper respiratory tract. Second, the G-form does not appear to cause more severe disease. Third, G-form spikes were more infectious than the ancestral D-form. Then the scientists showed that the G-form is neutralized by host antibodies *more*, not less, as might have been expected from its increased infectivity compared to the D-form. These findings all have a structural explanation.

The team's simulations revealed that indeed G-form spike proteins should have considerably more protomers in the up position at any given moment, about 75 percent, compared to 50 percent for D-form. Interestingly, the RBD is also a target for natural antibody-based neutralization, so the "more up" hypothesis also provides an explanation for the finding of increased neutralization.

The location of amino acid position 614, however, is not very near the RBD; it lies about halfway down the spike protein. How can a change at that distal location affect the molecule's likelihood to take the "up" shape? The computer models suggest that the D614G substitution acts by rearranging



Early in the COVID-19 pandemic, a new variant of the causative virus emerged. Instead of an aspartic acid (D) as the 614th amino acid in the spike protein, the new variant has a glycine (G) at that location. Over about three months, the G-form of SARS-CoV-2 displaced the original D-form as the globally dominant variant. The single amino acid substitution seems to confer increased infectivity in the upper respiratory tract, increased production of new virus, and increased neutralization by host antibodies.

There are around 200 SARS-CoV-2 vaccines in various stages of development.

Laboratory structural biologist Gnana Gnanakaran, who works with Korber, wanted to pursue that structural explanation. "Why is the virus taking the G-form?" he asks. "What is the function of that single amino acid substitution?"

A team of postdocs from Gnanakaran's group—Rachael Mansbach, Srirupa Chakraborty, and Kien Nguyen—ran molecular-dynamics simulations on the Lab's high-performance computers, simulating every atom of the spike protein in both D-form and G-form. Each spike is a trimer—a group of three identical molecules, or protomers, that work in concert as one functional unit. In electron micrographs, these protomers usually have their terminal region, or head, folded down, but occasionally one will have its head sticking up. The "up" configuration exposes a section of the spike protein called the receptor binding domain (RBD), which interacts with host-cell receptors to allow viral entry. So, more protomers in the "up" position would allow more binding and more entry into host cells.

hydrogen bonds within and between protomers, which relieves strain caused by the "up" position. In D-form, one protomer in the "up" position creates asymmetric interactions further down the molecule, forcing neighboring protomers into the "down" position. But in the G-form, the asymmetry is relaxed, resulting in a higher proportion of spike proteins with at least one protomer "up." Taken together, this amounts to the G-form being more infectious and more transmissible than the D-form. The theorists' findings have been confirmed experimentally by collaborators.

The D614G change is just one amino acid substitution; viruses like SARS-CoV-2 undergo this kind of substitution frequently throughout their structural proteins. Some substitutions are consequential, like D614G, but many aren't. Understanding these types of evolutionary mechanisms is crucial for vaccine design.

At the time of this writing, there are around 200 SARS-CoV-2 vaccine candidates in various stages of development, and preliminary results show great promise. In addition to providing structural modeling and viral evolution expertise to other vaccine designers, Korber and her team have two vaccine candidates of their own in the works. One is based on the spike protein and attempts to capture the natural diversity of the virus in key antibody targeting sites, so as to maintain efficacy as the virus evolves. The other will operate through a non-antibody immune mechanism whereby cells of the immune system track down and kill virus-infected cells. It's unclear how crucial this pathway is for SARS-CoV-2 immunity, but it's likely to be important because it can help resolve infection by the other highly pathogenic coronaviruses, Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) and Middle East Respiratory Syndrome Coronavirus (MERS-CoV).

SARS-CoV and MERS-CoV emerged earlier this century, in 2002 and 2012 respectively. Three highly pathogenic coronaviruses emerging in two decades highlights the need for a broad coronavirus vaccine—one vaccine that protects against many related viruses. Korber, her Los Alamos colleagues, and the broader NVBL consortium are keeping this in their sights so that the work they do for the current pandemic can help protect humanity during the next one.

Treating disease

In addition to designing vaccines to prevent infection, Korber and colleagues are also helping design therapeutics for treating COVID-19. One method of treating an infection is to administer exogenous antibodies—that is, antibodies not made by the patient's own body. These could be convalescent antibodies, which come from people who have survived the infection, or they could be artificially synthesized. Convalescent antibodies seem to be an effective treatment, but not one that is likely to be broadly available, so synthetic antibodies need to be developed.

"In May, when we started this work, we couldn't get many SARS-CoV-2-specific antibodies," says computational immunologist Kshitij Wagh. "So we used antibodies from the first SARS, SARS-CoV, as a starting point and began computationally designing variants of these that might be effective against SARS-CoV-2."

Unfortunately, SARS-CoV-specific antibodies don't protect against SARS-CoV-2—survivors of SARS are not immune to COVID-19. But Wagh and Korber understand the critical biophysical interactions between antibody and virus that make such antibodies effective against one but not the other. They are using this knowledge to design antibody variants that can improve interactions with SARS-CoV-2.

The antibody work that Wagh and Korber are doing for the coronavirus pandemic is underpinned by the many years of work they have done, and are still doing, for the *other* pandemic burning across the globe—HIV.

"HIV has been and remains a pandemic. It's slow burning, but it's still raging," says Wagh. He and Korber built their antibody-modeling expertise through their pursuit of therapies for HIV. They believe, as do many in the HIV field, that the key to COVID-19 antibody therapy is to make it a cocktail—a mix of at least two or three different antibodies. Naturally arising mutations in the virus could allow it to escape neutralization by one antibody, but it would be exponentially less likely to evolve two or three escape mechanisms at once. Artificially synthesized antibodies have the advantage that they can be tailored and tweaked to alter functionality, and one such tweak on the Los Alamos team's radar is to make an effective cocktail against other coronaviruses, not just SARS-CoV-2.

"It's quite likely that there will be another crossover event," emphasizes Wagh. "We've seen three in the last 18 years."

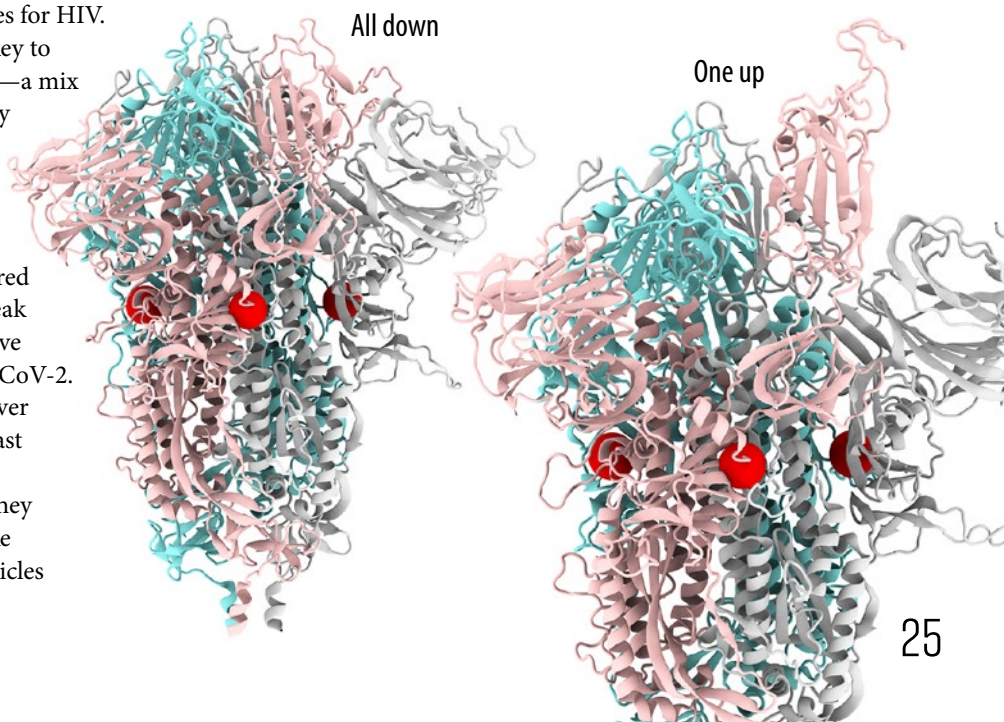
Antibodies can be very particular in the targets they recognize. If they are specific to "up" configured spike proteins, they would preferentially bind to virus particles with "more up." So Gnanakaran, in collaboration with colleagues at Duke University, is figuring out how to stabilize a spike protein in the "up" configuration, so that it might be used as an immunogen—a molecule against which antibodies are made. He is also studying the interaction between the spike protein's RBD and the human cell receptor, angiotensin converting enzyme 2 (ACE2), to see exactly where molecular recognition occurs.

It's quite likely that there will be another crossover event. We've seen three in the last 18 years.

ACE2 is important to the COVID-19 picture in more ways than one. It's the main molecule that SARS-CoV-2 uses to infect a human cell, but it has a normal job too. ACE2 is a key player in a complex blood-pressure and electrolyte-regulation pathway.

Sofiya Micheva-Viteva is a microbiologist at Los Alamos who is studying what happens when the virus, by binding to ACE2, prevents ACE2 from acting in its normal capacity. Ordinarily, when blood pressure dips, ACE2 helps bring it back up by producing vasoconstrictors and other cell-protective molecules. However, when ACE2 is bound by the virus, it can't do its job, and inflammation results, which can be pathogenic on its own and, in the case of SARS-CoV-2 infection, might exacerbate the symptoms of COVID-19.

Rather than live virus, Micheva-Viteva uses virus-like particles (VLPs), which are essentially empty virus particles; they have the same external proteins, including the spike protein, but there is no genome inside so they are incapable of replication. VLPs can bind to live cells in a mock infection, so scientists can safely study what happens within those cells during infection.



SARS-CoV-2 spike protein structural diagram showing three identical protomers (aqua, pink, and white) with location 614 (red) indicated for each one. (Left) The "all down" conformation shows the receptor binding domain of all three protomers lying flat. (Right) The "one up" conformation, with the receptor binding domain of the pink-colored protomer sticking up, is required for the virus to bind to and infect a host cell. *CREDIT: Kien Nguyen and Gnana Gnanakaran*

In particular, Micheva-Viteva is looking at a preexisting but still unlicensed drug. The drug is a synthetic version of one of the molecules that ACE2 is responsible for activating, and it has anti-inflammatory effects. If SARS-CoV-2 has bound to ACE2, then the anti-inflammatory molecule can't be activated, so inflammation and oxidative stress will rise. But the artificial version, the drug molecule, might be able to act in its place to restore regulation and minimize downstream damage. Any virus that binds ACE2 will interfere with this pathway, but this non-virus-specific therapeutic could be an effective way to restore function.

Rather than an anti-viral approach, Micheva-Viteva is pursuing a pro-host approach, a way to improve the patient's ability to handle infection. Though it wouldn't prevent infection, the therapy may dampen the severity of the disease enough to make it non-life threatening and keep the patient out of intensive care.

The receptor ACE2 doesn't act alone to let SARS-CoV-2 into a cell. ACE2 is how the virus knows it's in the right place, but to get in requires several other host-cell molecules. One of these is called "transmembrane protease, serine 2," or TMPRSS2. This molecule is an enzyme found on the cell surface, whose normal function is not entirely known. During SARS-CoV-2 infection, the enzyme acts on a particular piece of the virus's spike protein, breaking the amino acid chain at that spot. This is part of a process called priming, which increases the spike protein's structural flexibility and allows the membranes of the virus and cell to fuse.

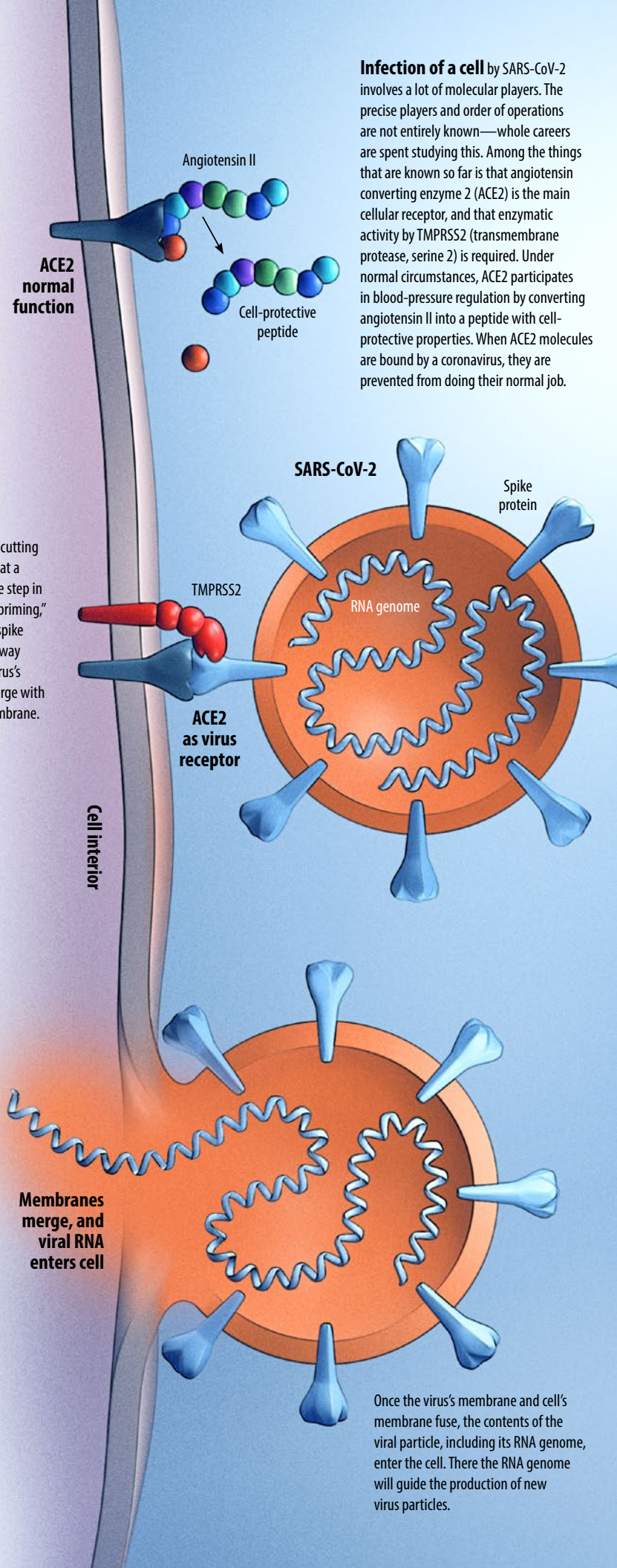
Los Alamos biophysicist Julian Chen is looking closely at the role of TMPRSS2, with an eye on disrupting its function.

"In order for a coronavirus to infect a cell, there are a lot of different steps," says Chen. "If we have a molecule that can interfere with any given step, that might be a drug that will work. There are many different points at which the process might be stopped."

To design a good TMPRSS2 inhibitor, Chen and colleagues need to start with an accurate structure of the TMPRSS2 catalytic domain, that is, the part of the protein that acts on the spike protein.

While an experimental structure for TMPRSS2 is not currently available, computational methods can produce a highly accurate model for TMPRSS2 by using available structures of similar proteins. Through computational modeling, Chen and collaborators have begun exploring what kinds of molecules would theoretically make a good inhibitor. And the winning molecule doesn't necessarily have to be something that prevents TMPRSS2 from cutting a protein; it could be something that TMPRSS2 cuts *instead* of the spike protein.

"We've made a short list of general candidates with certain desired chemical properties that are being tested theoretically," Chen explains. "Candidates that meet theoretical criteria will then be tested experimentally—we'll synthesize them and see how well they really work."



Infection of a cell by SARS-CoV-2 involves a lot of molecular players. The precise players and order of operations are not entirely known—whole careers are spent studying this. Among the things that are known so far is that angiotensin converting enzyme 2 (ACE2) is the main cellular receptor, and that enzymatic activity by TMPRSS2 (transmembrane protease, serine 2) is required. Under normal circumstances, ACE2 participates in blood-pressure regulation by converting angiotensin II into a peptide with cell-protective properties. When ACE2 molecules are bound by a coronavirus, they are prevented from doing their normal job.

TMPRSS2 acts by cutting the spike protein at a specific place, one step in a process called "priming," which alters the spike protein in such a way as to allow the virus's membrane to merge with the host-cell membrane.

Once the virus's membrane and cell's membrane fuse, the contents of the viral particle, including its RNA genome, enter the cell. There the RNA genome will guide the production of new virus particles.

Chen was always interested in viruses and was in high school when HIV came to prominence. He has worked on HIV therapeutics and points out that HIV therapy was transformed by the advent of drug cocktails designed to act on several different viral targets at once. This notion of hitting multiple targets is one of the major lessons learned from the past 30 years of HIV research and drug design. Now medical science is applying that concept to other viral infections, like SARS-CoV-2, to design multi-target treatment regimens. And TMPRSS2 is involved in cell entry not just for SARS-CoV-2 but also for SARS-CoV, MERS-CoV, and some influenza viruses, so drugs to inhibit its action could be included in a variety of therapeutic cocktails.

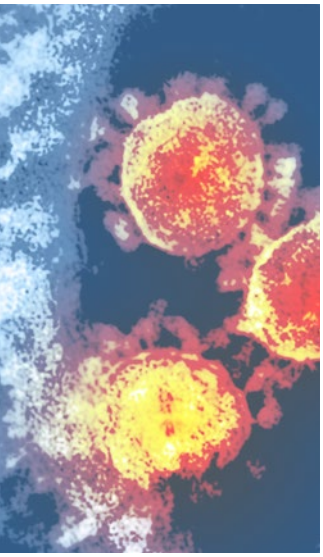
Making molecules

Whether trying to prevent infection or treating disease, computational molecular design goes hand in hand with synthesis of the actual molecules. Here too, Los Alamos scientists are leading the charge.

Los Alamos biochemist Ryszard Michalczyk oversees several molecular-synthesis projects. One of these is looking at drugs already approved by the Food and Drug Administration to see if any might be effective against COVID-19.

"We started with approved drugs because if one of them works, then the path forward is largely paved already," explains Michalczyk. "In collaboration with other national labs, we are screening thousands of compounds, using simulation to see how well they would work, then ranking them according to expected efficacy. Any molecules deemed worthy of further experimentation are then either purchased, when possible, or synthesized at Los Alamos."

"The NVBL molecular-design project is huge," adds Iyer. "Some of the molecules being looked at can be purchased commercially, but many cannot. Los Alamos has always had a strong organic-synthesis capability, and we are doing nearly all of the synthesis for the NVBL."



A digitally colorized and highly magnified transmission electron microscopic image of a close relative of SARS-CoV-2, Middle East Respiratory Syndrome coronavirus (MERS-CoV) at the cell membrane.

CREDIT: National Institute of Allergy and Infectious Diseases (NIAID), used with permission.

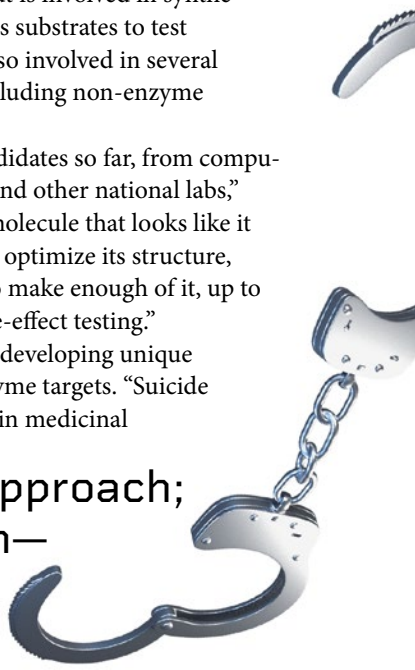
As molecular structures come in from computational designs, Laboratory chemists look at them from a synthesizability standpoint, to determine the best strategy, then begin making them. Sometimes the path is straightforward, having been previously published; other times there is no recipe, and the chemists have to invent one. Once the molecules are made and purified, they are sent to collaborating labs for testing, the results of which will inform subsequent iterations and improvements.

Many targets for therapeutics are enzymes, like TMPRSS2. In order to test whether the enzymes' actions have been affected, the scientists need substrates—the molecules that enzymes bind to and modify in some way—so they need to synthesize those too.

Los Alamos chemist Jurgen Schmidt is involved in synthesizing small molecule peptides to use as substrates to test the activity of various enzymes. He's also involved in several other molecular-synthesis projects, including non-enzyme drug candidates.

"We've got over 300 promising candidates so far, from computational predictions being done by us and other national labs," Schmidt says. "When we get a hit—a molecule that looks like it will do what we want it to—we have to optimize its structure, affinity, and selectivity, then we have to make enough of it, up to gram quantities, to do toxicity and side-effect testing."

Schmidt and collaborators are also developing unique suicide inhibitors for various viral enzyme targets. "Suicide inhibition" is a common method used in medicinal chemistry. It involves giving an enzyme a substrate that it can't get rid of, thus preventing any further action by that enzyme molecule. The trick is to make a substrate analog that preserves the affinity between substrate and enzyme, while simultaneously adding chemical groups that will cause the substrate and enzyme to bind irreversibly to one another.



This isn't an anti-viral approach; it's a pro-host approach—a way to improve the outcome of infection.

In yet another approach, Schmidt is also synthesizing peptides to directly help prevent and treat infection. He synthesized several peptides that mimic the region of the spike protein's RBD that is the most antigenic—the region most recognized by host antibodies. The synthetic peptides were then mixed with SARS-CoV-2-positive human serum, which recognized and reacted with the synthetic peptides. This result is preliminary, but it suggests small synthetic peptides may represent a viable vaccine strategy, or a first-response treatment measure.

Looking ahead

Scientists across the globe are working around the clock to develop vaccines and drugs to end the COVID-19 pandemic. Never before have so many minds been seated at the same table, working on the same problem.

Los Alamos brings to that table not just brilliant minds but established capabilities and world-class facilities. The Laboratory excels at computational molecular design, rapid chemical synthesis, and on-demand manufacturing of custom targets, and it is now leveraging these resources to help solve the global crisis.

The first world-changing vaccine came when Edward Jenner followed a hunch. The next one will be no less world-changing, but will be much more elegantly designed. LDRD

—Eleanor Hutterer

MANUFACTURING

NEW TOOLS FOR THE TOOLBOX

Reimagining what we need to solve supply shortfalls for this pandemic and avoid them for the next one

"NECESSITY IS THE MOTHER OF INVENTION," wrote Plato in *Republic*. Never have these words felt more apt than during a global crisis of historic proportion. Los Alamos National Laboratory was founded in such a time, and on a similar principle; the Lab's original mission—to build the first atomic bomb—centered heavily on invention. The success of that mission set Los Alamos on a track of unwavering commitment to innovation and invention.

Today the world faces a different kind of crisis. The coronavirus pandemic and the myriad of challenges that have sprung from it have brought scientists and inventors clamoring to help. Through innovation in manufacturing, they are finding ways of increasing supplies, such as safety equipment and test kit components, to meet a ballooning demand, and they are looking for ways to help meet the demand with the supplies on hand. They are inventing new designs, testing new materials, and generating vital data so that new solutions can be brought to market as quickly as possible.

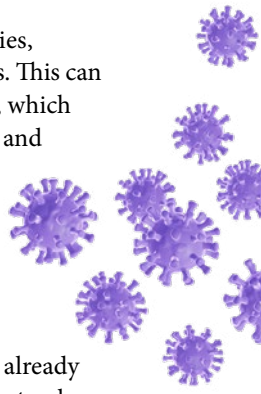
Innovators at the Laboratory are driven by professional commitment, personal circumstance, and global altruism. They are addressing all aspects of the problem, from preventing and detecting to treating and surviving infection by the novel human coronavirus, SARS-CoV-2. Here are some of the manufacturing projects that Laboratory scientists are working on to help with this pandemic and the next one.

Reducing the risk

"MAKE IT LIKE A DISHWASHER," George said, "a dishwasher for N95s—I think it has to be that easy."

It was April, 2020, and chemical engineers George Goff and Alex Marchi were brainstorming designs for a machine to decontaminate disposable N95 respirators using hydrogen peroxide vapor. Goff's wife is a healthcare worker and there was serious concern in his house—and across the nation—that supplies of disposable personal protective equipment, or PPE, would run out. (N95s are colloquially called "masks," but because they are engineered to form a seal on the wearer's face and to prevent at least 95 percent of particles larger than 300 nanometers (nm, billionths of a meter) from passing through, N95s are not mere masks but true respirators.)

Hydrogen peroxide is an unstable, oxidizing species, meaning it easily strips electrons from other molecules. This can result in the formation of highly reactive free radicals, which can irreparably damage the virus's envelope, proteins, and RNA genome.

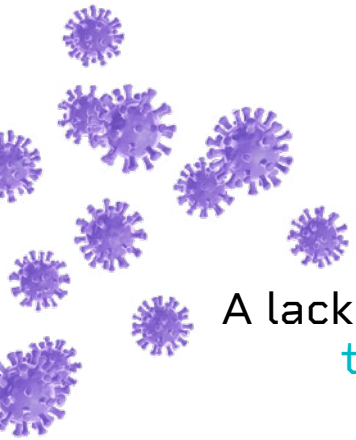


Smaller hospitals need smaller-scale solutions.

Similar in concept to how large commercial units already operate throughout the country, Goff's essential idea is to place many used N95s into a sealed container, saturate them with vaporized pathogen-killing hydrogen peroxide, then purge the toxic fumes and *viola!* Sterile mask, ready for reuse.

Whereas the large commercial units are great for the urban areas they serve, smaller cities and smaller hospitals need smaller-scale solutions. Unlike the urban units, which are shipping-container sized, take eight hours to sanitize a batch of a few thousand masks, and have trouble tracking which mask was whose, Goff and Marchi wanted their system to be much smaller, handle about a hundred masks at a time, and complete a cycle in just two hours. Furthermore, the commercial units rely on external supply channels for hydrogen peroxide—channels that fluctuate wildly in both cost and availability—while Marchi and Goff are building their system to generate its own.

The work draws on the considerable fuel-cell expertise that exists at the Lab. Fuel cells for cars, say, take oxygen (O_2) and hydrogen (H_2) and convert them into water (H_2O). Hydrogen peroxide (H_2O_2) is an unwanted byproduct in fuel-cell chemistry, so it is a perennial challenge to keep its production to a minimum.



Therefore, a lackluster fuel cell, one that makes too much H_2O_2, seemed like a good place to start to build an H_2O_2 generator.

"I called them up and said, 'Give me your worst fuel cells—I mean the real stinkers,'" recalls Goff.

The team's fuel cell-based H_2O_2 generator takes H_2O and O_2 (eventually perhaps plain air) and combines them into H_2O_2. As of this writing, it is producing H_2O_2 concentrations of about 7 percent (in water), which may seem low but the goal is just 10 percent, so it's almost where it needs to be. The problem, suspects Laboratory fuel-cell scientist Rod Borup, may be decomposition. H_2O_2 decomposes easily—both light and metal will cause it to break down (that's why it's sold in dark plastic bottles). The team is searching for hidden causes of decomposition within their system and is confident that the 10 percent threshold is within reach.

A lackluster fuel cell is a good place to start to build a hydrogen peroxide generator.

Also from prior fuel-cell work at the Laboratory come excellent custom chemical sensors. Commercial H_2O_2 vapor sensors are expensive, short-lived, and reach their lower limit of detection exactly at the OSHA upper limit of one part per million, above which it is considered unsafe to breathe. Goff and Marchi wanted a cheaper, longer-lasting, more sensitive sensor and found it in the adaptation of Laboratory carbon dioxide sensors that had been previously developed for fuel cells.

"The sensors really are central," explains Marchi. "You need to know that your N95 has really been decontaminated—that the chamber reached the necessary concentration and maintained it for the right amount of time—but equally important is the need to know, when you take it out, that you aren't breathing in residual harmful vapor."

The team is following Goff's vision of making it like a dishwasher: It's about the same size; the masks nest like bowls into pullout racks; and the user closes the door, pushes the button, and two hours later fresh masks can be distributed to their wearers.

This project is a collaboration that is bigger than just Marchi, Goff, and Borup; it brings together experts in fuel-cell chemistry, gas-sensor technology, 3D printing for prototype production, and fluid dynamics within the vaporization chamber. Although the panic over possible PPE shortages has relaxed a bit since April, the team believes it's still worth bringing this project across the finish line. First of all, the pandemic isn't over. Second, sooner or later it will happen again. Finally, and perhaps most poignantly, even in non-pandemic times, healthcare workers need reliable PPE, so having safe ways to reuse existing items helps curb waste while minimizing reliance on sudden influxes, whenever the need arises. ■

ON THE OTHER SIDE OF THE LAB another team is working on an entirely different way of sanitizing used N95s and other PPE for reuse.

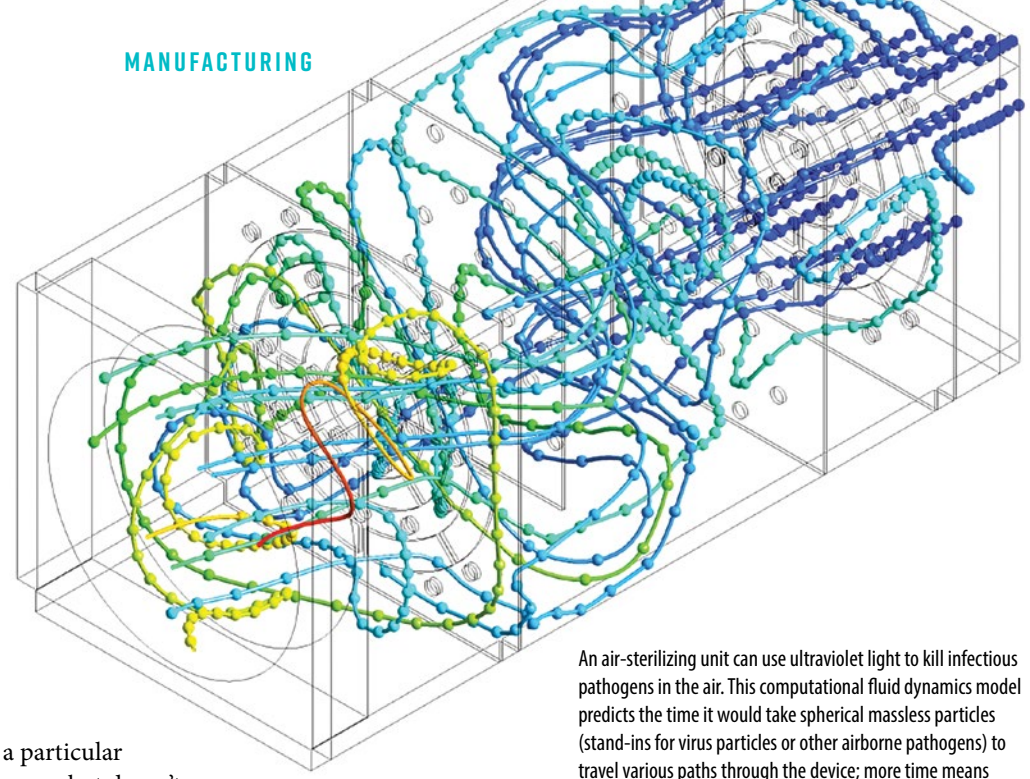
"Our idea is to use ionizing radiation," explains Los Alamos microbiologist Kumkum Ganguly. "It's not a new idea; people have used gamma rays before. But we are uniquely situated here, and we are doing it differently."

Ganguly and her Los Alamos colleagues Paul Peterson, Yongqiang Wang, David Seagraves, and James Hunter are taking advantage of the Laboratory facilities for nondestructive testing and evaluation, and for radiation protection services, to both eliminate infectious virus from PPE and study the effects, if any, the treatment might have on the materials. (Ganguly works with live coronavirus and collaborates on several other projects included elsewhere in this article to test inventions for virus-killing capacity. See "Testing the Tools" on page 33.)

First, they expose coronavirus-contaminated materials to either high-energy x-rays or gamma rays, then they compare the two methods to see which is best. Beyond comparing virus-killing capacity, the team is using computed tomography images and scanning electron microscope images to look at what structural changes, if any, the radiation causes in the PPE material. They are also determining changes in the materials' electron-charge retention and filtration capacity to identify any functional changes caused by the radiation.

Both x-rays and gamma rays of sufficient intensity will quickly damage the virus's genetic material beyond repair, rearranging chemical bonds within the molecule so that it becomes dysfunctional and even breaking the viral RNA into pieces. The scientists are doing a range of dose-determination processes, and Ganguly predicts that fairly low doses will be adequate to decontaminate PPE for reuse. This work reflects a unique collaboration between the Lab's bioscience, materials science, and weapons science divisions, all working together on the same platform to fight the pandemic. The team is also collaborating with Microsoft Research for further development. ■

BUT IT'S NOT JUST PPE that Laboratory scientists are decontaminating. Mechanical engineer Graham Arinder is working on a machine to clean the very air. Pre-pandemic, Arinder was working mainly on proton radiography and additive manufacturing projects, but when the pandemic hit, he and his colleagues began thinking of ways they could help.



An air-sterilizing unit can use ultraviolet light to kill infectious pathogens in the air. This computational fluid dynamics model predicts the time it would take spherical massless particles (stand-ins for virus particles or other airborne pathogens) to travel various paths through the device; more time means more sterilizing ultraviolet exposure.

"In an enclosed space, aerosols will build up over time, even if everyone is wearing a face mask. It's not realistic for everyone to wear respirators all the time," explains Arinder. "So, when someone sneezes or coughs in a conference room or shared office or lab, we want to be able to clean the air in that room."

There are two ways to do this: filtration and sterilization. Filtration removes particles above a particular size threshold, including pollen, dust, and pathogens, but doesn't kill them. Sterilization kills but doesn't remove airborne pathogens like viruses, bacteria, or fungi; the particles remain in the air but they are no longer infectious. Filters have to be monitored and regularly replaced, and in the era of coronavirus they are considered hazardous waste, demanding special disposal.

"From an operational standpoint," says Arinder, "sterilization makes more sense than filtration. This was an area of research before the pandemic, but now it's been fast-tracked to get it usable."

Arinder's air sterilizer is basically an airtight box, about 20 cubic feet in volume, full of ultraviolet (UV) light bulbs (similar in appearance to the fluorescent tubes in office overhead lighting), with an air-handling system to move the air through. The air is drawn in, passed through a gauntlet of UV light where any pathogen's genetic material is irreparably damaged, and blown back into the room once sterile.

The length of time that air spends inside the unit is a balancing act: Fast circulation produces better turnover but is less efficient at killing airborne viruses, while slower circulation kills the virus more effectively but leaves infectious virus lingering in the air for longer. Arinder believes the sweet spot is about 7–10 full air changes per hour. This is similar to the standard that hospitals use for filtration of air in patient areas.

The air sterilizer could be installed permanently or used on a temporary basis because it doesn't require any building retrofitting or special ducting—it sits on casters and can be rolled into any room where it's needed. The technology is also scalable to almost any room size. Furthermore, the cost of occasional light-bulb replacement is a fraction of the cost of regular filter replacement for air-filtration systems. Arinder is still fine-tuning both airflow and UV intensity; for this he is collaborating with scientists at Sandia National Laboratories and students at Texas A&M University, who are working on similar systems to decontaminate the air coming out of COVID-19 patients' ventilators.

Arinder never thought he'd be inventing an air sterilizer, just as Ganguly hadn't planned on irradiating PPE, and Goff and Marchi didn't know they would be building a dishwasher for N95s. But in a global emergency like this, when decontamination suddenly matters like it has never mattered before, innovators across the Lab and around the world look at their skill sets and ask themselves, "How can I help?" ■

Changing the conversation

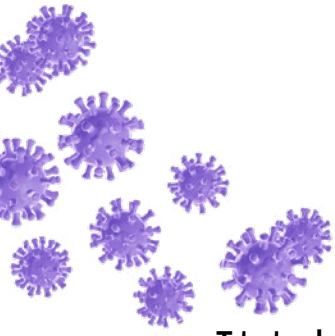
"EARLY ON, THE MATERIALS NEEDS WERE CHANGING ALMOST CONTINUOUSLY," recalls Laboratory chemical engineer Matt Lee, who oversees several manufacturing efforts aimed at remedying shortfalls and shortcomings of single-use consumable supplies.

"Depending on the day, one might hear anything from 'We need ventilator parts! We need face shields!' to 'Wait, never mind, now we need test swabs!' Everyone was eager to help," Lee continues. "We were spinning our wheels a bit; it was hard to make a clear plan that could have tangible impacts. But then we realized that instead of reacting to the news of the day, we should be anticipating the news of tomorrow."

Filtration removes viruses but doesn't kill them; sterilization kills them but doesn't remove them.

One project Lee and others are working on is the development of a virucidal respirator: a reusable respirator that not only filters the air to keep pathogens out but actually kills pathogens that come into contact with it.

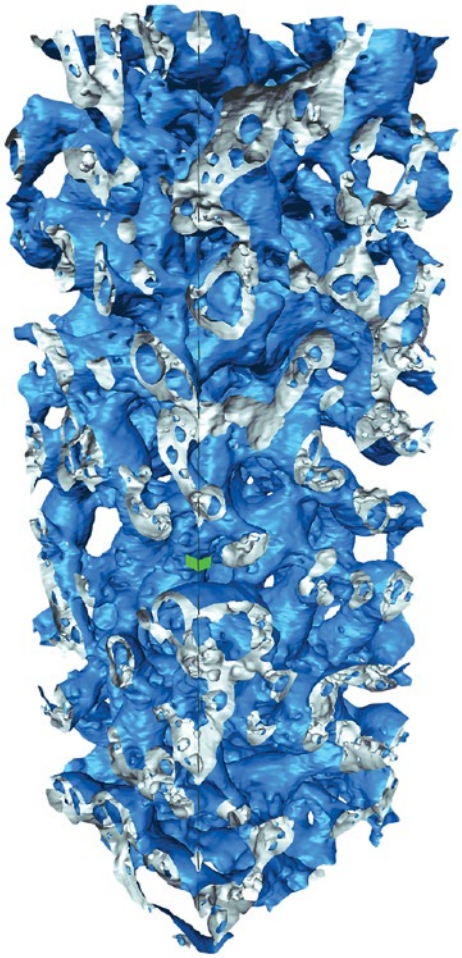
"Our goal is to make reuse a better option," explains Lee's collaborator, Laboratory electrical engineer Nina Weisse-Bernstein, who leads the project. "When a healthcare worker



is faced with a shortage of PPE and has to make the choice of whether to reuse a mask or take a fresh one from an ever-dwindling supply, the use of virus-killing materials within the PPE itself makes reuse far less risky."

N95 respirators, whether disposable or reusable, rely on fiber-based filters to prevent infectious viruses from passing through; fibers are layered randomly over one another until a certain level of occlusion is achieved. Weisse-Bernstein is developing a reusable respirator with a filter that not only traps pathogens but kills them because the filters are made of copper.

It takes 4 hours on a copper surface for 95 percent of coronavirus to be killed.



A 3D rendering of a copper nanofoam. Copper is naturally antimicrobial and could be used as a filter for self-sterilizing respirators. Scientists are using the Lab's 3D-printing capabilities to fine tune the pore size and distribution within the nanofoam.

The natural ability of copper to kill pathogens on contact has been appreciated by the medically minded for nearly 7000 years; however, the exact mechanism by which it does this is still unclear. Perhaps copper ions disrupt the electrical potential across a microorganism's membrane, or maybe they recruit damaging reactive-oxygen species, or they may interfere with the transcription and translation of the pathogen's genetic material, or maybe the effect is a combination of these activities. In any event, it takes four hours on a copper surface for 95 percent of infectious coronavirus to be killed; by the end of a 12-hour shift, any virus that came into a nurse's respirator filter during the first eight hours would almost certainly be dead.

Weisse-Bernstein's team is using 3D printing to produce copper nanofoams. A prototype is 3D-printed from a mixture of copper and plastic polymer, then the polymer is baked away, leaving just the copper in a foam-like configuration.

Foam has different morphology than stacks of fibers, so a foam-based N95 will have a different airflow. The scientists are studying the airflow through various versions of copper foam to see how breathability is affected. With the 3D-printing method of manufacture, the number, size, and density of open pores can be tuned to achieve the best combination of filtration capacity and breathability. The variable that seems to matter the most in terms of breathability is the width of the copper material in between the pores, while filtration capacity is most affected by pore size.

The copper filter will attach to reusable N95 respirators. Such an antimicrobial option for N95s would mean filters don't need to be changed nearly as often and would also drastically reduce the need for disposable N95s. Because it's not coronavirus-specific, this technology would protect healthcare workers against other respiratory pathogens too. Though driven by present-day needs, it could truly be a paradigm shift for the future of respiratory PPE. ■

ANOTHER 3D-PRINTING-BASED PROJECT at the Lab addresses shortages of consumables used for detection and treatment of infection. Testing and treatment involve many single-use items, from swabs and tubes to ventilator hoses and hose adapters; if any one of those supplies runs out, testing or treatment could be stymied. So, Lee and colleagues are exploring ways of making them faster, cheaper, or reusable.

"The Laboratory is researching what could happen if a particular material supply chain is disrupted, and we're looking at ways to address those problems," explains Lee. "We aren't manufacturing consumable products in large supply per se, but rather developing new manufacturing technologies and designs that are more robust to those disruptions."

Laboratory scientists build prototypes for new concepts and do the science to prove the prototypes work. Once the proof-of-concept work is done, the Lab hands it off to partners in industry to bring the products to market.

The Laboratory's 3D-printing capability is a key resource enabling rapid design and prototyping. Alex Marchi (of the N95 dishwasher project above) is a 3D-printing expert and is working with Lee to rapidly produce and test prototypes from new designs and new materials for single-use supplies. She is making things like plastic tubes for testing reagents, flow regulators for ventilators, sticks and packaging for nasal swabs, and more.

Marchi makes these items using stereolithography—a 3D-printing technology wherein plastic is laid down in liquid form, then UV light is used to solidify and bind the layers together to form a specific object. Marchi is exploring different kinds of plastic as well as different designs for making faster and cheaper consumables. If the item is to be reusable, then it also needs to be able to withstand decontamination methods, like heat, bleach, hydrogen peroxide, or ionizing radiation.

It's pretty straightforward to imagine how some consumables, like swabs, tubes, and hoses, might be used in a clinical setting.

But Los Alamos scientists are also developing other, very particular items whose utility can only be conceived by those in the trenches, the healthcare workers treating COVID-19 patients. ■

EDITH DANIELSON is a hospital respiratory therapist who is married to Laboratory physicist Jeremy Danielson. In early 2020, Edith and her colleagues were warned to expect a shortage of ventilators, the life-support machines that keep unconscious patients breathing.

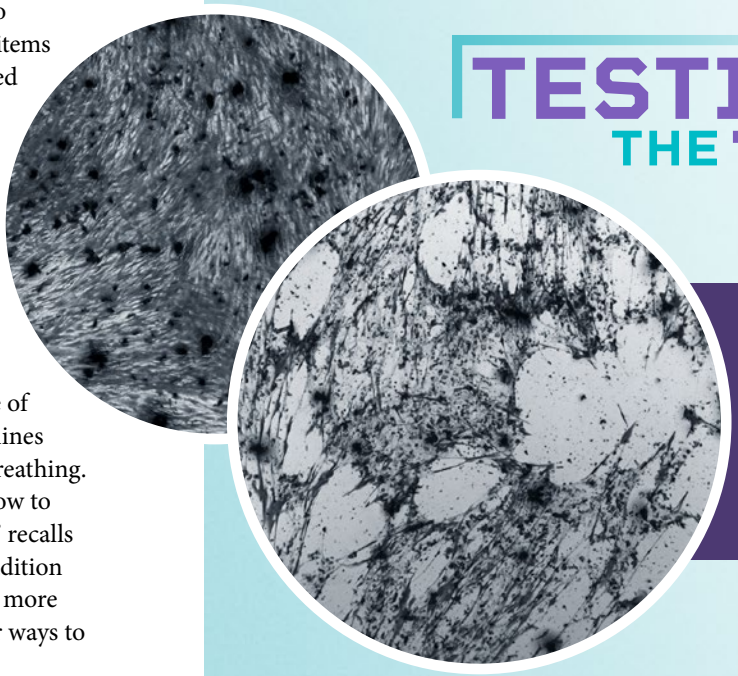
"We sat down to talk about how to prepare for the coming shortage," recalls Edith, "and we decided that in addition to trying to meet the demand for more ventilators, we wanted to look for ways to reduce the demand as well."

Ventilators aren't the only machines that help people breathe—positive airway pressure (PAP) machines are prescribed to thousands of people each year to help treat sleep apnea. Edith knew that PAP machines are much more abundant than ventilators, and it seemed to her like a promising option if and when the shortage arrived.

The challenge with using PAP machines to help COVID-19 patients breathe is that these machines have exhale valves as well as emergency anti-asphyxiation vents that aren't filtered, so the machine and the entire room would quickly fill with exhaled coronavirus. The Danielsons, along with Edith's manager at the hospital, Daniel "Scotty" Sylva, teamed up with Lee and Los Alamos electrical engineer Jeremy Payton, and the group began discussing ways of retrofitting a filter onto a PAP machine. Viral filters for breathing tubes exist, but the diameter for the filters doesn't match the diameter of PAP machines' breathing tubes, so it was a matter of making an adapter to fit them together. Laboratory research technologist Ruben Manzanares was brought in to help turn rough ideas into actual parts that could be tested.

As the pandemic progressed, the team began seeing more reasons to choose PAP over ventilation. First, a patient needs to be sedated for ventilation and can't be taken on and off as needed. Second, the needs of COVID-19 patients, in terms

TESTING THE TOOLS



Left: Human lung cells are long and thin and form a continuous monolayer with all of the cells a relatively uniform size. Right: Infection is apparent when holes appear in the monolayer. The holes form when infected cells die, losing adhesion from their neighbors and bursting to release thousands of new viruses.

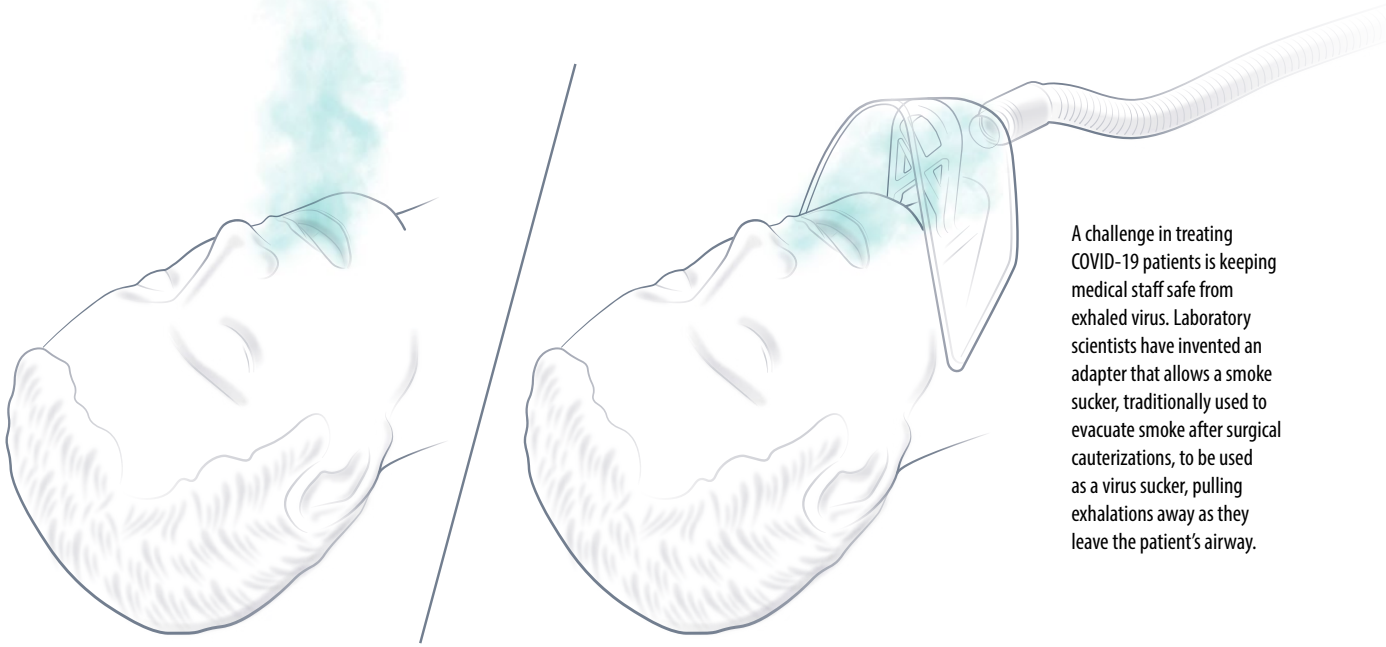
LOS ALAMOS MICROBIOLOGIST KUMKUM GANGULY works with scientists to determine if and how well their machine or material kills coronaviruses. For items like Arinder's air sterilizer or Weisse-Bernstein's copper filter, it is crucial that the virus truly be eliminated as expected. But for other things, like Marchi's 3D-printed test components, it's vital that the material *not* kill the virus, lest the test report a false negative.

Los Alamos is not equipped for work with live SARS-CoV-2, so Ganguly and her team, Samantha Adikari and Seychelles Voit, use less pathogenic surrogates: two human coronaviruses, termed HCoV-229E and HCoV-OC43, both known causes of the common cold, that are structurally similar to SARS-CoV-2.

The scientists culture human lung cells in plastic dishes containing a specialized liquid growth medium. When a coronavirus infects a cell, the cell becomes a virus-making factory, and when it becomes too full of virus, the cell bursts and releases thousands of new virus particles into the surrounding growth medium. The team uses this virus-containing medium to determine different items' virus-killing capacity. After coating an item, say a small piece of novel polymer, in a known quantity of virus and incubating for a specific amount of time, the item is washed with fresh medium that will then be given to fresh, uninfected cells. If the new cells become infected, they will begin to die after a few days and the scientists know that either the item itself or the incubation time was insufficient to kill all of the virus.

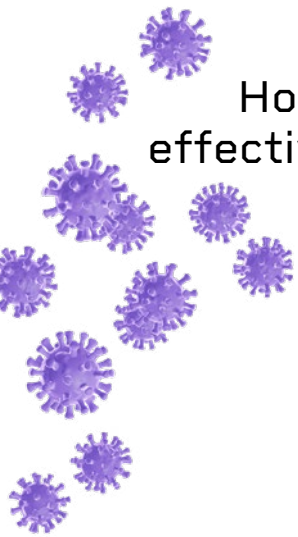
Ganguly's team also does reciprocal tests, called biocompatibility tests, which determine if coming into contact with the virus affects the material and, if necessary, how the material stands up to common sterilization methods. And because the materials have to be effective against any pathogen that a healthcare worker or patient might encounter in a hospital setting, the team does bacteria-killing and bacteria-growing tests as well.

Finally, once a new invention passes muster in Ganguly's lab, it still has to be proven against actual SARS-CoV-2. For this Ganguly will collaborate with scientists at other institutions with special labs designed for safe handling of this dangerous virus.



A challenge in treating COVID-19 patients is keeping medical staff safe from exhaled virus. Laboratory scientists have invented an adapter that allows a smoke sucker, traditionally used to evacuate smoke after surgical cauterizations, to be used as a virus sucker, pulling exhalations away as they leave the patient's airway.

of air pressure and flow, tend to fluctuate dramatically, and PAP machines are more nimble than ventilators for these kinds of adjustments. Third, it was becoming apparent that ventilators weren't necessarily improving patients' outcomes. Ventilators aren't meant to be used for more than a few days, but COVID-19 patients can be kept on for up to 60 days, during which time their lungs may be damaged not only by the infection but by the ventilator itself. So the team started thinking about PAPs not as ventilator stand-ins, but as ventilator preventatives—a way to circumvent the vents.



Homemade masks are surprisingly effective for relevantly sized droplets.

The invention is an adapter that needed to be simple, easy to use, and made from nontoxic material that can be sterilized (an early version melted in the autoclave). The team tried different designs and different materials and finally arrived at something that worked.

"The adapter is a relatively simple part," says Jeremy Danielson, "but it's a part that the medical community asked for, and we are happy to supply a design."

And the team didn't just make one adapter, they made two.

Another problem that Edith and Scotty saw while treating COVID-19 patients arose during intubation. Inserting a breathing tube into a patient's trachea is a potentially messy procedure, made dangerous by the coronavirus. COVID-19 patients have to be intubated with their upper bodies inside a clear plastic glovebox to protect the medical staff, but it makes a difficult procedure even harder. Edith wanted something to suck the air away from a patient's face, thereby creating a small area of negative air pressure, so that a medical worker could quickly intubate the patient without inhaling any of the patient's exhaled air.

This type of machine exists; it's called a smoke evacuation unit and is used during surgery to evacuate smoke after cauterization, for example.

"If you hook a face mask to a smoke sucker, to suck exhalations away," Edith explains, "the suction is too strong, and the mask sucks down onto the patient's face, blocking access to the airway."

The adapter the team made to turn a smoke sucker into a virus sucker is a plastic bridge that supports a face mask—the clear plastic kind used in hospitals to administer oxygen—by holding the face mask close to the patient's mouth, while keeping it out of the way. The team designed and fabricated two versions: one for emergency use and one that takes into account patient-comfort considerations for longer-term use.

This setup would be particularly good for hospitals with limited resources that lack negative-pressure rooms. Even small hospitals have built-in vacuum lines, which, with the addition of in-line virus filters, could safely be used to similar effect as a smoke-evacuation unit. The ability to create a negative-pressure zone would also be useful for other respiratory pathogens, like influenza or tuberculosis, or during the administration of toxic medication.

"This is one of the most fulfilling projects I've worked on," says Payton, who, before the pandemic hit was working on subcritical nuclear weapons experiments at the Nevada National Security Site. "When Edith brought these ideas to us, we started looking at them right away. It felt good to be helping the hospital workers directly."

The team has provided prototypes of both inventions to a local hospital for evaluation within its laboratories, where the data needed for Food and Drug Administration (FDA) approval and industry partnerships will be collected. ■

Delivering the data

FOR A NEW DEVICE, MATERIAL, OR DESIGN to receive FDA approval, it needs to come with a litany of performance data. For some pandemic-related inventions, the design is done but the data are lacking. Here too, Laboratory scientists are helping by designing and conducting experiments to provide the missing numbers.

For example, early in the pandemic there was a lot of discussion about whether laypeople should wear masks, and if so, what kind and under what circumstances.

"Do masks actually make sense?" asks Laboratory physicist Michael Ham. "It helps to have data to inform those types of decisions, so we did the experiments."

Ham and colleague Yong Tao use a cough machine, designed by Laboratory mechanical engineer and airflow expert Murray Moore, to evaluate how well various types of material might help contain the expelled virus. What kind of homemade mask would have the best filtration capacity: Bandana? T-shirt? Fancy bedsheets?

The cough machine sprays droplets of fluorescent liquid onto a homemade mask with a piece of filter paper behind it to catch whatever passes through. Then the proportion of droplets that are caught on the mask is compared to the proportion on the filter paper. The smaller the droplet, obviously, the more likely it is to go through, but also, and perhaps less obviously, the less likely it is to be infectious.

A SARS-CoV-2 particle is about 0.1 micron in diameter (a micron, or µm, is a millionth of a meter), and N95 respirators are effective at filtering down to about 0.01 µm. Speaking produces droplets of about 1 µm, and coughing produces droplets of about 200 µm. It has been estimated that a 10-µm droplet has a 37 percent chance of containing a single virus particle, so while coughing produces droplets large enough to contain at least one virus, speaking usually doesn't. The infectious dose, or the number of SARS-CoV-2 particles it takes to successfully infect a person, is difficult to determine, but scientists suspect it might be as low as a few hundred. The team concluded that homemade masks are actually quite effective for the most relevant droplet sizes.

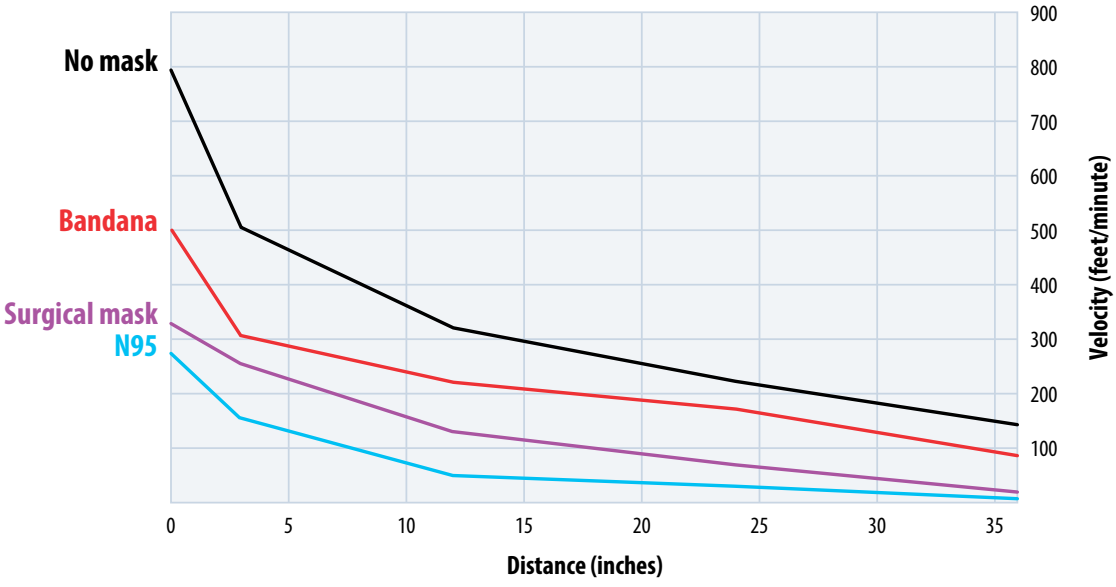
"There's an argument I've heard that a cloth mask keeps SARS-CoV-2 out as well as a chain-link fence would keep a mosquito out," Ham explains. "But that's a false analogy in most instances. You're looking to catch droplets, not single viruses, so it would be more like using a chain-link fence to keep out a trash bag full of mosquitos."

The scientists found that if two people are wearing homemade masks and standing in pre-pandemic proximity, about 50 percent of respiratory droplets produced by Person 1 will be caught by Person 1's mask, while 50 percent of the droplets that passed through will be caught by Person 2's mask. So only 25 percent of the droplets leaving Person 1's airway might enter Person 2's airway, and both masks participate in reducing the risk for both people. This factor-of-four reduction can be further reduced if the two people keep a larger distance between themselves than they would have done during pre-pandemic times, so that most of the droplets leaving Person 1 will fall to the ground before reaching Person 2.

The exception to this is healthcare workers. Medical staff who are spending prolonged periods of time in confined spaces with confirmed, highly infectious patients will receive inadequate protection from homemade masks. Single SARS-CoV-2 particles can linger in the air and accumulate to a dangerous concentration in such a circumstance. So medical workers absolutely need N95s to do their jobs safely, but for the rest of us, homemade masks will do for picking out produce at the grocery store.

In addition to the cough-machine tests, the team did a series of particle transport tests using a wind tunnel that Moore had previously built at the Lab for air-quality testing.

Team member Rebecca Williams, who is an industrial hygienist and the Laboratory's PPE subject-matter expert, explains, "As forward velocity changes by talking or breathing, the filtering capabilities of the masks can change as well. Expelled air contains moisture, and as the day goes on, the mask can accumulate and retain that moisture, causing it to be weighed down, which can change how the mask fits, and thus its filtering efficiency."

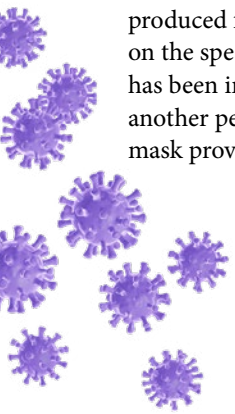


Laboratory scientists tested how well various types of face coverings impede forward air velocity of particles coughed out by a cough machine. All materials tested provided some impedance, with N95 respirators providing the most, and a double-folded bandana providing the least.

The scientists studied forward air velocity and particle permeation through various materials to determine how novel materials would perform for all-day wear. The team also evaluated which materials would best impede forward air velocity, limiting one person's breath from reaching another person's breathing zone. For example, they determined that air exhaled while speaking without a mask travels fast enough to register on an anemometer at 10 feet, but only 2 feet with a mask. They also found that cloth masks remain effective for up to six hours in the wind tunnel.

Using a pseudo-saliva made from water, glycerin, and table salt, the team did evaporation tests. They found, for example, that a 5-μm droplet traveling at 1.5 meters per second will fully evaporate during its 20-foot flight from generator to detector. This matters because evaporation quickly reduces the size of droplets, correspondingly reducing their filterability.

So outside of a healthcare setting, virus-containing droplets produced from speaking and breathing are very likely to be caught on the speaker's mask. If a droplet does go through, its velocity has been impeded so it won't travel as far, but if it does reach another person, and that person is also wearing a mask, their mask provides another chance to catch the droplet.



Knowing how to best use our tools is going to help determine best practices for treating COVID-19.

"We found that masks do work to stop the spread," says Ham. "I think it would help consumers understand this if store-bought masks were to have ratings that tell their filtering capacity and breathability. Our data ought to help that happen." ■

ANOTHER PROJECT THAT IS HELPING PROVIDE necessary data is a ventilator testbed established by Laboratory engineer Todd Jankowski. Earlier this year, when the country was expecting to run out of ventilators, inventors began designing low-cost, easy-to-source medical respiratory devices, casually dubbed "DIY ventilators." Not actually DIY ("do it yourself"), in the sense that one can't put oneself on a ventilator—it requires full sedation and a medical team—the DIY ventilator designs use parts found at hardware stores and are intended for hospitals that are overwhelmed by COVID-19 patients in need of breathing assistance.

Jankowski didn't design a DIY ventilator; he designed a rig that can test how well a DIY ventilator performs. The ventilator testbed and its personnel do three things for a DIY design: they test the device as designed, refine the design, and produce detailed, easy-to-use assembly instructions. First, Jankowski and his team build the DIY ventilator according to the designer's instructions, then they outfit it with pressure, flow, and oxygen sensors and collect performance data. While building and operating, the engineers and technicians on the team look for possible design improvements and suggest solutions if problems are encountered. Once the design is final, they write and illustrate detailed, user-friendly assembly instructions. Armed with the test data and the assembly instructions, the inventor can now apply for a patent and FDA approval. ■

ALONG SIMILAR LINES to the ventilator testbed comes another Laboratory project that is looking at a specific type of commercial ventilator to understand how it might be used for COVID-19 patients. Intrapulmonary Percussive Ventilators (IPVs) pulse fine aerosols into a patient's lungs to help break up and move mucus. IPVs are typically used to treat patients with severe asthma or premature infants whose lungs aren't functioning properly.

"We wanted to study the science and engineering of the IPV because it's never been used before for COVID-19," says Laboratory engineer John Bernardin, who leads the project. "What we're trying to study is how the aerosol generated from an IPV affects mucus that resides in an infected lung. Does it help clear a path for air to get in?"

The experimental setup is a 3D-printed model of the first three branches of the human bronchotracheal system (there are 23 branches in all). The model is equipped with various sensors to determine how air moves down lung passages and how it changes in terms of concentration and droplet-size distribution. An artificial lung is attached to the model, and as it breathes in and out, the researchers can see how the aerosol changes. There are also optical sensors that can watch if and how a patch of lab-grade mucus, applied to the inside of the 3D-printed model, is affected by the IPV.

The data from these and other experiments, as well as data produced by computational fluid dynamics modeling, are used to train a machine-learning algorithm that can predict the effect of various IPV treatment settings. Knowing how to best use the tools that we already have, as well as inventing new tools, is going to help the medical community determine best practices for treating COVID-19. ■

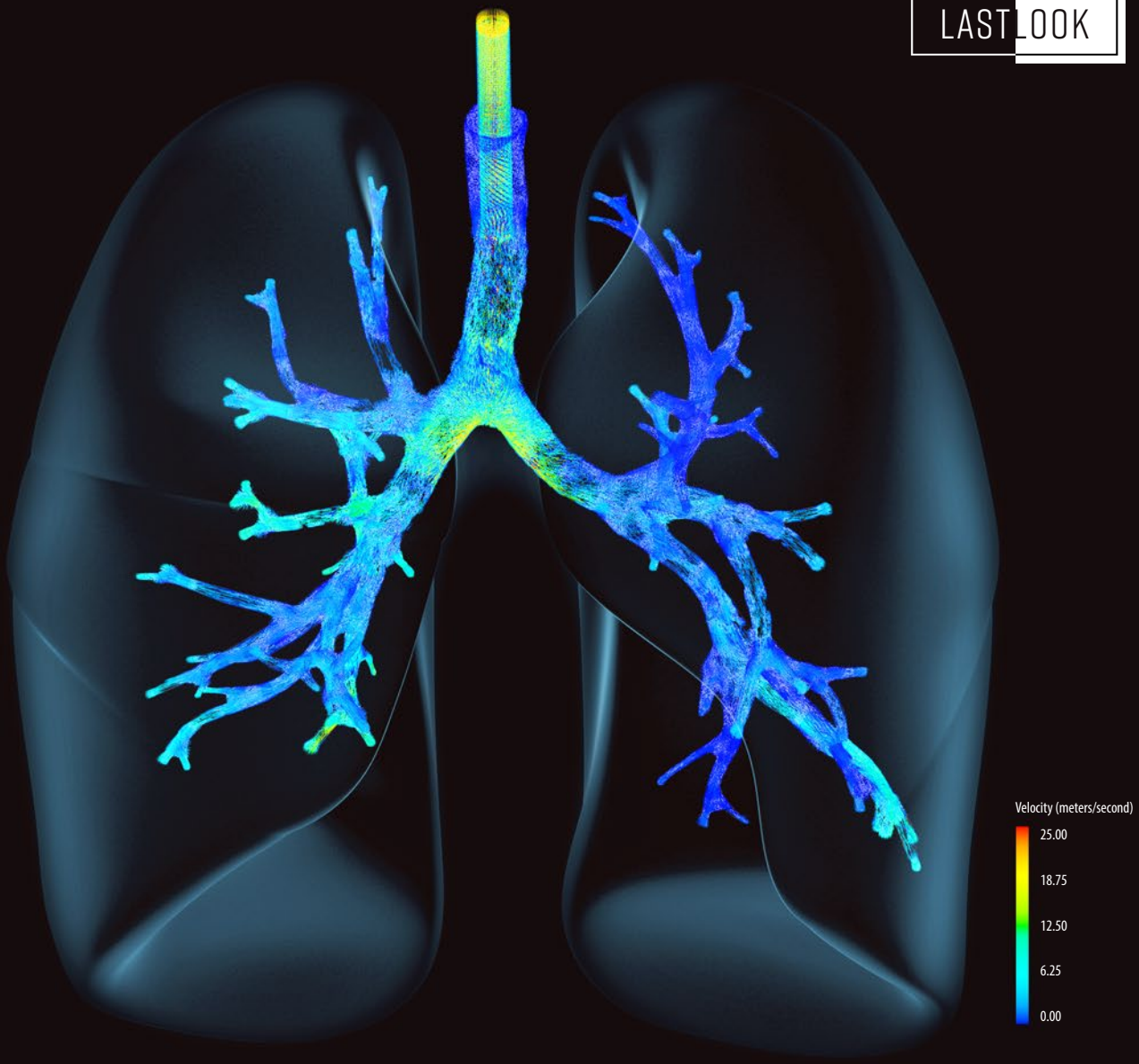
Readying the resources

Collaboration lies at the heart of scientific endeavor, especially now when the world is united in the common cause of ending the coronavirus pandemic while saving as many lives as possible. Many of the projects described here are larger than Los Alamos; they are national multi-institution collaborations established to solve pandemic problems quickly and permanently.

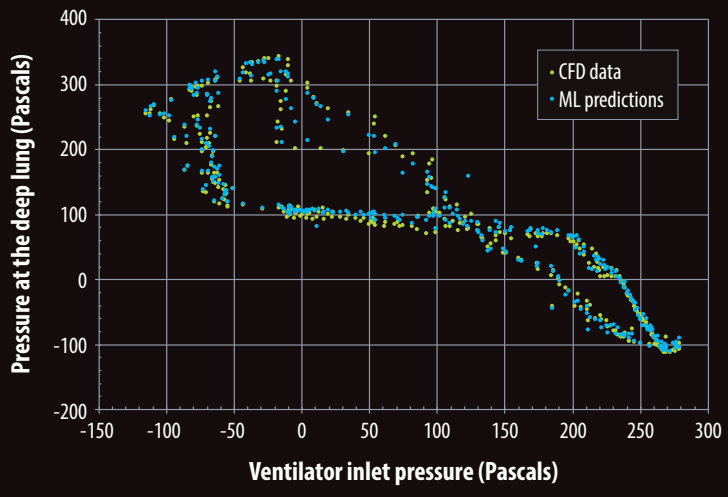
Lab scientists are working to ameliorate supply shortages by finding new ways to produce things and inventing altogether new things. From gas sensors to ventilator adapters to new kinds of PPE, Los Alamos designs and data will help the Laboratory's partners in industry bring vital new technologies to market.

It takes time, effort, and expertise to get new technologies going. Racing against the clock and achieving success under the gun is something the Laboratory has specialized in ever since its first mission. Now, as then, when the stakes are highest, Los Alamos scientists are answering the call. LDRD

—Eleanor Hutterer



Above: Laboratory scientists are studying how different ventilator types and settings affect what happens within the lungs. For example, this mathematical visualization shows the velocity of air entering the lungs from high-frequency oscillatory ventilation, which rapidly pulses small volumes of air instead of the "inflate-deflate" cycle of a conventional ventilator. In this example, the machine is set to 15 cycles per second with a peak flow of 60 liters per minute, which is the upper bound of what a patient would typically receive with this type of treatment. The research includes experimental work as well as computational fluid dynamics (CFD) modeling, both of which provide data for a machine learning (ML) algorithm. For more about the experimental work, see "New Tools for the Toolbox" on page 28.



Right: CFD data and ML predictions are in strong agreement in this chart showing air pressure in the deep lung as it relates to ventilator pressure settings. CFD calculations can't be done in real time, so a rapid, efficient, and accurate hybrid ML tool is being developed to provide doctors with real-time input for patients undergoing mechanical ventilation due to COVID-19 or other respiratory diseases.

Credit: John Bernardin, Jacob Riglin, and Arvind Mohan, LANL.

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